M Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population

A DISSERTATION SUBMITTED TO THE TAMIL NADU Dr. M.G.R. MEDICALUNIVERSITY, IN PARTIAL FULFILMENT OF THE REGULATIONS FOR THE AWARDOF M.D. DEGREE IN PAEDIATRICS EXAMINATION TO BE HELD IN MAY 2018.

CERTIFICATION

This is to certify that this dissertation entitled " ***Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population.***" is the bona fide original work of Dr. Praveen George Paul under the guidance of Dr. Sarah Mathai, Professor, Department of Paediatrics,Christian Medical College,Vellore, towards partial fulfillment of university regulations for the award of M.D. Paediatrics Degree examination of The Tamil Nadu Dr. M.G.R. Medical University, Chennai to be held in May, 2018.

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INTRODUCTION

Congenital hypothyroidism (CH) is the commonest preventable cause of mental retardation in children. Diagnosis and initiation of Thyroxine supplements as early as possible after birth, preferably within the first two weeks of life is imperative to prevent neurocognitive impairment. However the clinical symptoms and signs of congenital hypothyroidism take several weeks to manifest causing major delay in diagnosing this condition. The introduction of newborn thyroid screening and early initiation of Thyroxine supplements in children with congenital hypothyroidism have dramatically improved their neurocognitive outcome. Over the last two to three decades universal newborn screening(NBS) for CH is available in all the developed and some of the developing countries.

In Christian Medical College, Vellore, screening for congenital hypothyroidism has been implemented since July 2001. All babies born at our hospital above 26 weeks of gestational age have cord blood sent for TSH assay as part of standard of care. Those with cord blood TSH above the cut-off level are recalled for confirmatory sampling. A diagnosis of primary congenital hypothyroidism is made if TSH is elevated and Free T4 level is low for the age. In the initial 8 months of our screening programme, the cord blood TSH cut-off was 20 mIU/L. Using this cut-off level, none of the 5209babies screened were confirmed to have congenital hypothyroidism, therefore the screen cut-off was increased to 25mIU/L in April 2002 and has remained the same since then. Over the last 16 years we have confirmed primary congenital hypothyroidism in 123 babies. .

Unfortunately there is no national NBS programme for congenital hypothyroidism in India. Several private and government facilities have initiated their own newborn screening programmes. There is no consensus as to what is the most appropriate TSH cut off value with different centers using values ranging from 10mIU/L to 40mIU/L. Keeping in mind the devastating and irreversible adverse neurodevelopmental outcome of a delayed or missed diagnosis of CH in children, the need of the hour for India is to initiate universal NBS for CH with clearly laid out guidelines including screening cut-offs to facilitate early diagnosis and prompt initiation of therapy..

One of the road blocks to implementation of national newborn screening for congenital hypothyroidism may be its doubtful cost benefit ratio. In the light of our vast experience with a successful ongoing NBS programme, this study was proposed in order to identify an ideal cord blood TSH level which not only has high sensitivity as a screening tool for congenital hypothyroidism, but also has an acceptable recall rate.

LITERATURE REVIEW

INTRODUCTION

The development of the Thyroid gland is mediated and regulated by the coordinated expression of numerous developmental transcription factors like the thyroid transcription factor-1, thyroid transcription factor-2 and paired homeobox-8, which are expressed selectively in the thyroid gland.[1] The correct combination of the above factors result in thyroid cell development as well as the induction of thyroid-specific genes - thyroglobulin, thyroid peroxidase, the sodium-iodide cotransporter and the thyroid-stimulating hormone receptor.[2]

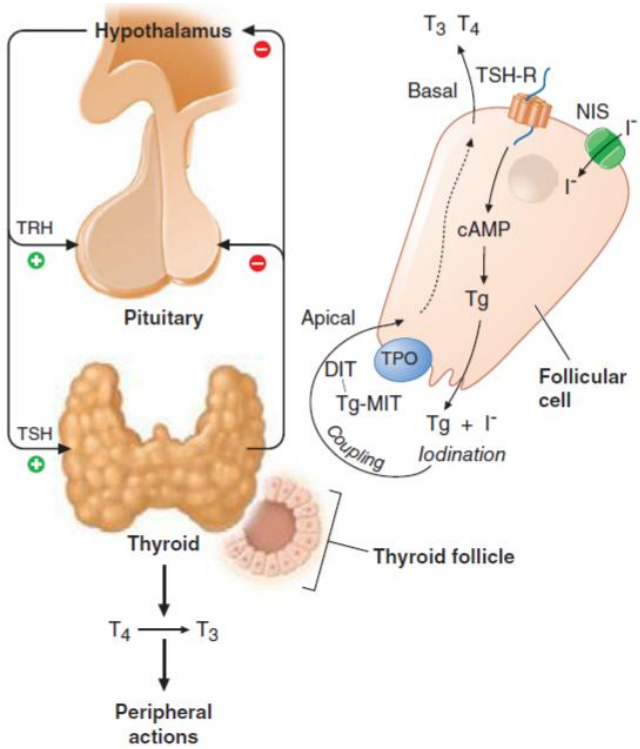
Mutations in the genes coding these transcription factors are responsible for rare causes of thyroid agenesis or dyshormonogenesis. However, the causes of most of the cases of congenital hypothyroidism remain idiopathic. The reported prevalence of CH in the developed countries has changed from 1:7000-10,000 prior to the NBS era, to 1:3000- 4000 (1970s, USA) to 1:2273(2007, US)[3].

However the prevalence is much higher in several other countries including 1:748 (Iran), 1:1600Pakistan)[4,5]. In India prevalence of CH is different in different states1:1700 (Hyderabad), 1:3400 (Chandigarh) and 1: 1700 (Lucknow)[6].

Hence universal newbornscreening is now the standard in most countries, as early thyroid hormone replacement in newborns with congenital hypothyroidism can prevent or minimise potentially severe developmental abnormalities associated with the illness.[7]

REGULATION OF THYROID FUNCTION

The thyroid axis is an endocrine feedback loop, where the levels of thyroid hormones in the body are closely regulated to maintain metabolic homeostasis. Thyroid releasing hormone (TRH) produced in the hypothalamus stimulates the production of the Thyroid Stimulating Hormone (TSH) which in turn will stimulate thyroid hormone synthesis and secretion from the thyroid gland. On the other hand, thyroid hormones provides a negative feedback predominantly through the thyroid- hormone receptor β2



(TRβ2) which inhibits TRH and TSH production (Figure 1).

Figure 1Regulation of thyroid gland (Adapted from Harrisons textbook of medicine, 19th edition)

The "set-point" in the above thyroid axis is established by TSH, which is secreted by the thyrotropes in the anterior pituitary gland and like other pituitary hormones is secreted in a pulsatile manner. It is composed of 2 subunits – α and β subunits – with the β subunits being unique to TSH; whereas the α subunit is common to other glycoprotein hormones like the follicular stimulating hormone (FSH), luteinizing hormone (LH) and Human chorionic Gonadotropin (hCG).

THYROID HORMONE SYNTHESIS

The follicular cells of the thyroid gland produce thyroglobulin, a large glycoprotein from which the thyroid hormones are synthesized. Thyroglobulin is then iodinated on tyrosine subunits which are then bound by ether linkages. The release of T3 or T4 into the blood stream results after thyroglobulin reuptake occurs by the follicular cells of the thyroid gland, within which proteolysis occurs as the final step.

Iodide uptake is the first and most critical step in the synthesis of thyroid hormones. Most of the iodine which is ingested is bound to albumin in the serum and constitutes the fraction which is absorbed by the body for thyroid hormone production. Unbound iodine is almost entirely excreted in the urine.

The thyroid gland can extract iodine from the circulation in a very efficient manner. Tracer studies show that up to 10–25% of radioactive iodine can be taken up by a normal thyroid gland over a 24 hour period, and this value can increase up to 90% in

disease states like Graves' disease. This process of Iodide uptake is mediated by Sodium iodide symporter (NIS), which is present at the basolateral membrane of the thyroid follicular cells. This symporter is most densely expressed in the thyroid gland, but low levels can be found in the membranes of the placenta, the lactating breasts and the salivary glands.

This iodide transport mechanism is also highly regulated that it allows a wide range of adaptation depending on the variations in dietary availability of iodine. Low iodine in the diet causes in an increase in the levels of Sodium iodide symporter (NIS) and results in increase iodide uptake.High iodine levels leads to suppression of NIS expression and subsequent iodide uptake. It is this selective expression of NIS in the thyroid gland that allows the use for radioisotope of iodine in isotopic scanning, treatment of hyperthyroidism, and radioisotope ablation of thyroid cancer and at the same time minimizing significant effects of the same on other organs of the body. Mutation of the Sodium iodide symporter (NIS) gene is a rare cause of congenital hypothyroidism.

Pendrin is another iodine transporter which is located on the apical surface of thyroid cells. It mediates the efflux of iodine into the lumen of the thyroid follicles. Mutation of the pendrin gene results in the Pendred syndrome – a congenital disorder which is characterized by defective organification of iodine, goiter, and sensorineural hearing loss. [8]

After iodide is absorbed into the thyroid gland, it is retained inside the cell and transported to the apical cell membrane of follicular cells of the thyroid gland.

Thyroxine peroxidase and hydrogen peroxide which is produced by dual oxidase(DUOX) and DUOX maturation factor (DUOXA) results in an organificationreaction in the follicular cells which oxidizes the iodide molecule to a reactive iodine atom. This combines to certain selected tyrosyl residues within the thyroglobulin which a large protein that is composed of 2769 amino acids. These newly produced iodo -tyrosines molecules in Thyroglobulin are then coupled to each other via an ether bond in a reaction that is again catalysed by TPO enzyme.

This reaction can lead to production of Either T4 or T3 depending on the number of iodine atoms present in the iodotyrosines molecules. Thyroglobulin can then be taken back into the thyroid cell, where it is processed in lysosomes present inthe cytoplasm to release T4 and T3. The resulting uncoupled mono- and iodotyrosines are then de-iodinated by the enzyme dehalogenase which recycles the iodide that has not been used for synthesis of thyroid hormones.

Defects in thyroid hormone synthesis can result in rare causes of congenital hypothyroidism. The majority of these disorders are a result of recessive mutations in TPO or Thyroglobulin. However, mutations have also been increasingly identified in the TSH receptor genes, pendrin, NIS, dehalogenase and hydrogen peroxide generation. The result of this biosynthetic defect results in the gland being incapable of synthesizing adequate amounts of thyroid hormones. This mostly manifest as congenital hypothyroidism with increased levels of TSH and a large goiter.

SITE OF ACTION OF THYROID STIMULATING HORMONE

TSH, a hormone produced in the anterior pituitary gland, is responsible for regulation of the thyroid gland function through its action on the TSH receptors (TSHR). The TSH receptor is a transmembrane G protein–coupled receptor (GPCR) which is coupled to the alpha subunit of stimulatory G protein (GSα), which in turn activates adenylyl cyclase and results in the increased production of cyclic adenosine monophosphate (AMP). TSH also activates phospholipase C which increases phosphatidylinositol turnover.

The functional role of the TSH-R is mainly seen by the effects of naturally occurring mutations of the TSH-R. Recessive mutations can cause loss-of-function of the receptor and cause complete thyroid hypoplasia and congenital hypothyroidism. On the other hand, dominant mutations can result in gain of-function mutations which are responsible for sporadic or familial hyperthyroidism - characterized by goiter, hyperplasia of follicular cells and autonomous T hyper-functioning thyroid gland.

A majority of these activating mutations takes place in the transmembrane portion of the TSH receptor. Such mutations can mirror the conformational changes that takes place as a result of TSH binding or the action of thyroid-stimulating immunoglobulins (TSI) that are seen in Graves' disease. These TSH-R mutations can also occur as somatic events which can result in clonal selection and multiplication of the affected thyroid follicular cell resulting in autonomously functioning thyroid nodules.

Although TSH is the most important hormonal regulator responsible for growth of the thyroid gland and its optimal functioning, other factors, which includes a variety of growth factors that are locally produced, can also influence Thyroid hormone synthesis, release and function. These factors includes insulin-like growth factor I (IGF-I), transforming growth factor β (TGF-β), epidermal growth factor, endothelins, and many other cytokines. However the quantitative roles of each of these factors are not yet fully understood. These factors are more important in the context of certain selected disease states like acromegaly, where there exist increased levels of the growth hormone and IGF-I levels. This is associated with enlargement of the thyroid gland and an increased predisposition to multi-nodular goiter (MNG).

THYROID HORMONE ACTION

Thyroid hormones which are in circulation in the blood stream enters the cells partly by passive diffusion and also via specific cell transporters like the monocarboxylate 8 transporter, the monocarboxylate 10 transporter and organic anion-transporting polypeptide 1C1. Mutations in the genes encoding these transporters have been identified in patients who present with X-linked psychomotor retardation and also in patients with thyroid function abnormalities.

After entering the cells, the thyroid hormones act mainly via nuclear receptors, although, they may also have non-genomic actions by stimulating mitochondrial enzymatic responses which may directly have actions on blood vessels as well as the heart, mediated by the integrin receptors.

Thyroid hormones bind to nuclear thyroid hormone receptors (TRs) α and β with very high affinity. Both these thyroid hormone nuclear receptors are expressed in almost all the tissues of the body, with their relative expression levels varying among different organs. Thyroid hormone receptor α is particularly found in large concentrations in the brain, the kidneys, the gonads, in muscle and heart as well. Thyroid hormone receptor β however is expressed relatively higher in the pituitary gland and in the liver. In the pituitary gland, the thyroid hormone receptor β plays a role in feedback control of the thyroid axis.

These nuclear receptors bind to specific sequences in the DNA called thyroid response elements (TREs), which can be found in specific regions of target genes. These receptors sometimes bind as homo- dimers or as heterodimers with retinoic acid X receptors. These activated receptors can then either stimulate gene transcription of myosin heavy chain α, or can also inhibit transcription of the TSH β-subunit gene, as per the nature of the regulatory elements in the gene of target.

ROLE OF THYROID HORMONE IN METABOLISM AND MYELINATION

Clinical and experimental studies so far had shown that thyroid hormone is vital to the development of the brain. Majority of these studies were done during the postnatal period. Recent studies have however shown that thyroid hormone is essential to brain development throughout the fetal period and that the timing and severity of the thyroid hormone deficiency can predict the type and extent of the neurological sequelae. Thyroid hormone insufficiency during the first half of pregnancy leads to defects in

visual processing, attention and gross motor skills. While thyroid hormone deficiency that occurs later in the pregnancy leads to subnormal visuospatial skills and fine motor developmental delay, deficiency that occurs in the postnatal period leads to defects in memory and language skills.[9]

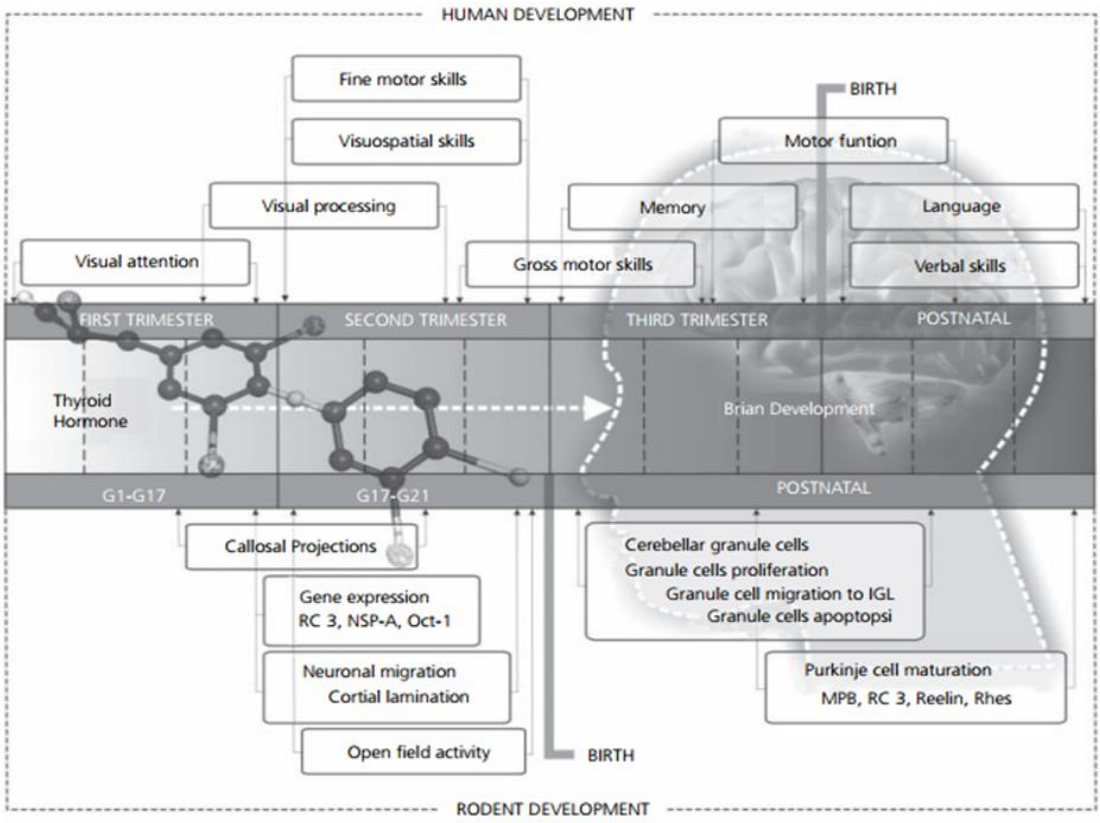


Figure 2. Figure showing the various defects in humans and rodents caused by thyroid hormone deficiency depending during various time period of fetal and post natal life.

(Adapted from Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol 2004;16:809–18)

ROLE OF IODINE IN THYROID FUNCTION

Iodine is a trace element present in low concentrations in the soil, air and sea.Iodine content of plant and animal foods reflects iodine content of local soil. Depletion of

iodine from the soil occurs due to soil erosion resulting from high rainfall or glaciation.

The dietary sources of iodine are marine foods such as fish, shellfish, seaweed , milk and dairy products, meat and eggs. With iodinisation of salt, iodised salt is one of the most important daily source of iodine.

In the human body, iodine is found in minute amounts and is an essential substrate for the synthesis of thyroid hormones[10]. Thyroid gland needs 52µg of iodine every day. Sodium/iodide transporter transfers iodine from serum to thyroid at concentration gradient of 20-50 times that of plasma.

The clinical manifestations of iodine deficiency reflect either the direct consequences of iodine deficiency on the thyroid or the secondary consequences of hypothyroidism on the thyroid hormone sensitive target tissues. If the requirements of iodine are not met, functional and developmental abnormalities including those of thyroid function occur. When the iodine deficiency is severe, endemic goitre and cretinism, endemic mental retardation, decreased fertility rates and increased perinatal death and infant mortality have been documented[11].

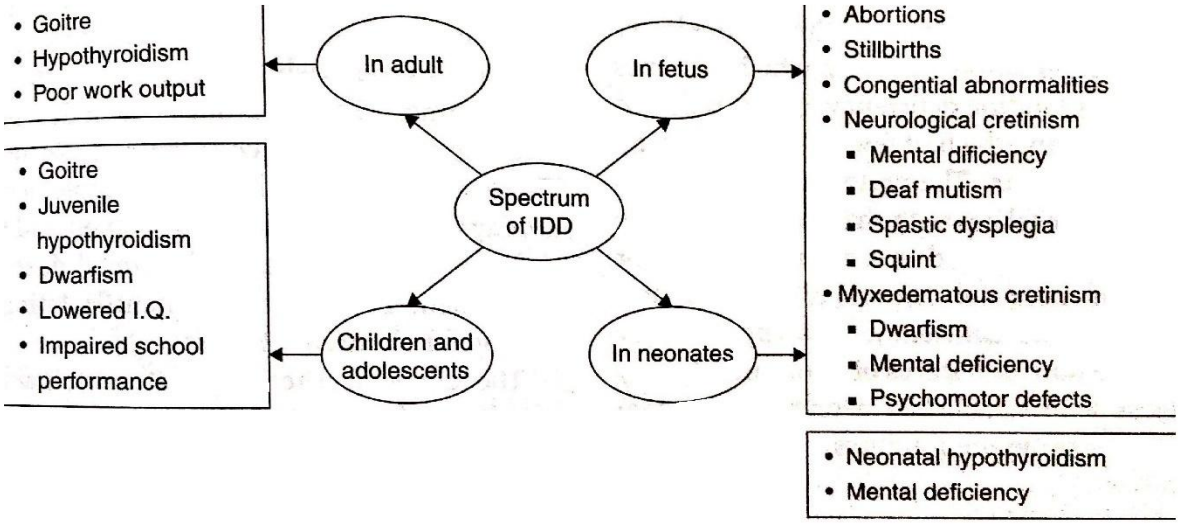


Figure 3: The spectrum of iodine deficiency disorders as adapted from Pediatric Endocrine Disorders. Desai, P M, Menon VB& PSN. Orient Blackswan; 2001.

Cretinism, the most severe form of permanent mental retardation, results from maternal iodine deficiency in early pregnancy.

Urinary iodine concentration is a reliable marker of iodine status in the body and is a gold standard for population studies. Urinary iodine concentration of 100-199 µg/L indicates adequate iodine status[12]

The variations in thyroid profile noted in individuals living in iodine deficient regions are decreased T4 or FT4, normal or increased T3, and a normal or increased TSH compared to the normal population. The neonatal TSH level is reported to be a more reliable indicator of iodine deficiency in the population with higher TSH levels during the first few weeks of life in the iodine deficient community. Delange et al stated that the most important and frequent alterations in thyroid function due to iodine deficiency occur in neonates and young infants in Europe[13]. This is substantiated by two important observations:

The risk of transient hypothyroidism in neonates is directly proportionate to the degree of prematurity[14]. The role played by iodine deficiency in this transient syndrome is supported by the disappearance of the same after supplementation with potassium iodide.

An inverse relationship was established between urinary iodine concentration in new born population in Europe, an index of state of iodine nutrition and frequency of serum TSH above 50 mU/ml at day 5[15]. In contrast, of the 71 Turkish newborns with urinary iodine concentration <100 mcg/L, only 48% of the term neonates with iodine deficiency had high serum TSH levels ≥11.2mIU/L[11]. These authors postulated that serum thyroglobulin levels may be a better indicator of Iodine deficiency.The thyroglobulin level indicates iodine status over a prolonged period.

The hypothyroid state due to iodine deficiency may be transient or permanent.A study conducted in Xinjiang, China revealed that the prevalence of both overt and subclinical hypothyroidism was higher in iodine deficient group[16,17].

IODIZATION OF SALT – IN THE INDIAN CONTEXT

Iodination of salt as prophylaxis for Iodine deficiency disorder began in the 1920s in the USA and Switzerland pioneered by Dr. David Marine in Akron, Ohio, USA This landmark study revealed that the administration of iodide tablets produced a decrease in the incidence of goiter in adolescents. This action was initiated in 1950s and 60s in Asia but gained momentum only by the late 1970s[18]

A landmark study was conducted in India in 1954 to establish the efficacy of iodination of common salt by the Government of India, the State Government of Punjab and the Indian Council of Medical Research in Kangra, Himachal Pradesh. The region was divided into three zones- A, B and C. After evaluating baseline characteristics, salt fortified with potassium iodide was given to those in Zone A and salt fortified with Potassium Iodate to Zone C. Zone B was provided salt without iodine. Analysis conducted in 1962, it became clear thshowed that the prevalence of goiter had reduced in zone A and C and hence iodine fortified salt wasplanned for all the zones.n. Second surveys done in 1968 and 1972 showedthat while zones A and C showed continuous decline in goitre prevalence , the prevalence of goiter in Zone B declined only after the provision of iodised salt in 1962. [19].

Based on the success of the Kangra Valley study, the Government of India launched the National Goitre Control Programme (NGCP) in 1962 which was 100% centrally assisted. The programme aimed to identify goitre endemic regions of the country and supplement the intake of iodide in these regions. It focused predominantly on the "goitre belt" which comprised the Himalayan and Tarai regions. It was later observed that IDD were reported from almost all areas of the country. Universal Salt Iodization (USI) as the preferred strategy to eliminate IDD was introduced in India in 1986. . Currently 91% of the Indian households have access to iodized salt and 71% consume adequate amounts[20].

CONGENITAL HYPOTHYROIDISM

Congenital hypothyroidism is one of the most common preventable causes of mental retardation in the pediatric population. The prevalence of congenital hypothyroidism as observed in western studies is about 1:4000[21]. However a large multi-centric study done by AIIMS across India screening over one lakh newborns between the year 2007 to 2012 found the prevalence to be much higher at 1:1221[22]. Our own data at Christian Medical College, Vellore has found the prevalence to be about 1:1200(unpublished data).

Screening for congenital hypothyroidism began in the early 1970s. The rationale for incorporation of screening for congenital hypothyroidism into the NHS(UK) was that they found improved CNS outcomes in those babies with CH in whom treatment was initiated by three months of life[23]. Through their newborn screening program, the NHS was largely able to eradicate the adverse neurodevelopmental outcomes that arise from congenital hypothyroidism[24]. It is only appropriate that India with its high prevalence of congenital hypothyroidism adopt a similar screening strategy so that this preventable cause of mental retardation can be diagnosed early and started on appropriate treatment, thereby reducing the morbidity and adverse outcome significantly.

GENETICS OF CONGENITAL HYPOTHYROIDISM

The inheritance of congenital hypothyroidism is generally sporadic. However in 2% of the cases of thyroid dysgenesis, the inheritance maybe familial and congenital hypothyroidism that is caused by organification defects maybe inherited recessively. The genes responsible for this genetically heterogenous disorder form two major groups: (i) those causing thyroid gland dysgenesis and (ii) those causing dyshormonogenesis. The genes associated with dysgenesis of thyroid include Gsα, TTF-1, TTF-2, Pax-8(thyroid transcription factors) and different syndromic complexes involving congenital hypothyroidism. Amongst the group causing dyshormonogeneis, the thyroglobulin and thyroid peroxidase genes were described initially. However in the recent past NIS(sodium iodide transporter), PDS(Pendred syndrome) and THOX2(Thyroid oxidase) gene defects are also described[25,26]. More recently, certain mutations in DUOX2 or THOX2(enzyme dual oxidase 2) have been detected. These lead to dyshormonogenesis due to deficient hydrogen peroxide generation and its inheritance has been found to be autosomal dominant[27].

Table 1: The genes responsible for congenital hypothyroidism

Gene	Thyroid	Other systems(few features)
TSH-R	Variable TSH resistance	
TTF-1	Hypothyroidism	Neonatal respiratory distress, hypotonia, choreoathetosis etc
TTF-2	Agenesis	Cleft palate,spiky hair
PAX-8	Hypoplasia	Activates WT1 gene
GNAS1	TSH resistance	Albright hereditary osteodystrophy
MCT8	↓ T4, ↑ T3 &↑TSH	Central hypotonia, nystagmus,feeding problem

CLINICAL FEATURES

The clinical features of a newborn with congenital hypothyroidism is often subtle and hence many infants are undiagnosed at birth[28]. This could be attributed to the passage of the maternal thyroxine through the placenta. The measured level of thyroxine in umbilical blood is found to be about 25-30% of normal[29]. Thus this thyroxine transferred from the mother has some protective effect on the fetal brain development[30]. The absence of such overt clinical signs and symptoms at birth, coupled with a newborn screening program that is restricted to large tertiary hospitals, it is important for clinicians to pick up these subtle clinical signs and symptoms which would help in early diagnosis and prevention of permanent neuro-developmental sequelae.

SYMPTOMS OF CONGENITAL HYPOTHYROIDISM

The initial symptoms of congenital hypothyroidism could be nondescript. However, the pregnancy and maternal history could provide some important clues. In about 20% of cases, the pregnancy may exceed beyond 42 weeks[28]. There could also be a history of maternal history of an autoimmune thyroid disease, a diet deficient in iodine or inadvertent treatment with radioactive iodine during pregnancy, which is rare. Once the baby is brought home, these are often found to be quiet and sleeping most of the day. There could also be symptoms such as hoarse cry, constipation and neonatal hyperbilirubinemia that lasts for more than three weeks[31]. The following table

illustrates some of the symptoms present in babies with congenital hypothyroidism during the time of newborn screening:

	Group 1 (n = 215) Initial T4 ≤ 30 nmol/L % with feature	Group 2 (n = 232) Initial T4 > 30 nmol/L % with feature
Prolonged Jaundice	59	33**
Feeding Difficulty	35	16**
Lethargy	34	14**
Umbilical Hernia	32	18*
Macroglossia	25	12*
Constipation	18	10
Cold or mottled skin	18	10
Hypothermia	3	3
No symptoms	16	33**
Other clinical features reported:		
Abnormal cry	7	6
Edema	5	3
Hypothyroid appearance	6	2
Hypotonia	3	3

**p < 0.001, *p < 0.01.

Figure 4: Prevalence of symptoms of congenital hypothyroidism at time of diagnosis (modified from: Alm et al. Brit Med J 289:1171-175, 1984 [13].)

SIGNS OF CONGENITAL HYPOTHYROIDISM

The common signs seen on initial examination of a baby with congenital hypothyroidism are macroglossia, umbilical hernia and dry mottled skin. Since the thyroid hormone is essential for the formation and maturation of bones[32], these babies could have wide open posterior fontanelles, usually greater than five millimeter. This along with poor feeding and persistent jaundice form the most

striking clinical feature[33]. In a minority of babies with dyshormonogeneis, where the defect is with thyroid hormone production, there may be a palpable goiter.

Figure 5: ***Infant with congenital hypothyroidism****. A - 3 month old infant with untreated CH; Picture demonstrating hypotonic posture,*

Infant with congenital hypothyroidism, myxedematous facies, macroglossia, and umbilical hernia. (adapted from LaFRANCHI S. Congenital Hypothyroidism: Etiologies, Diagnosis, and Management. Thyroid 1999;9:735–40. doi:10.1089/thy.1999.9.735.)

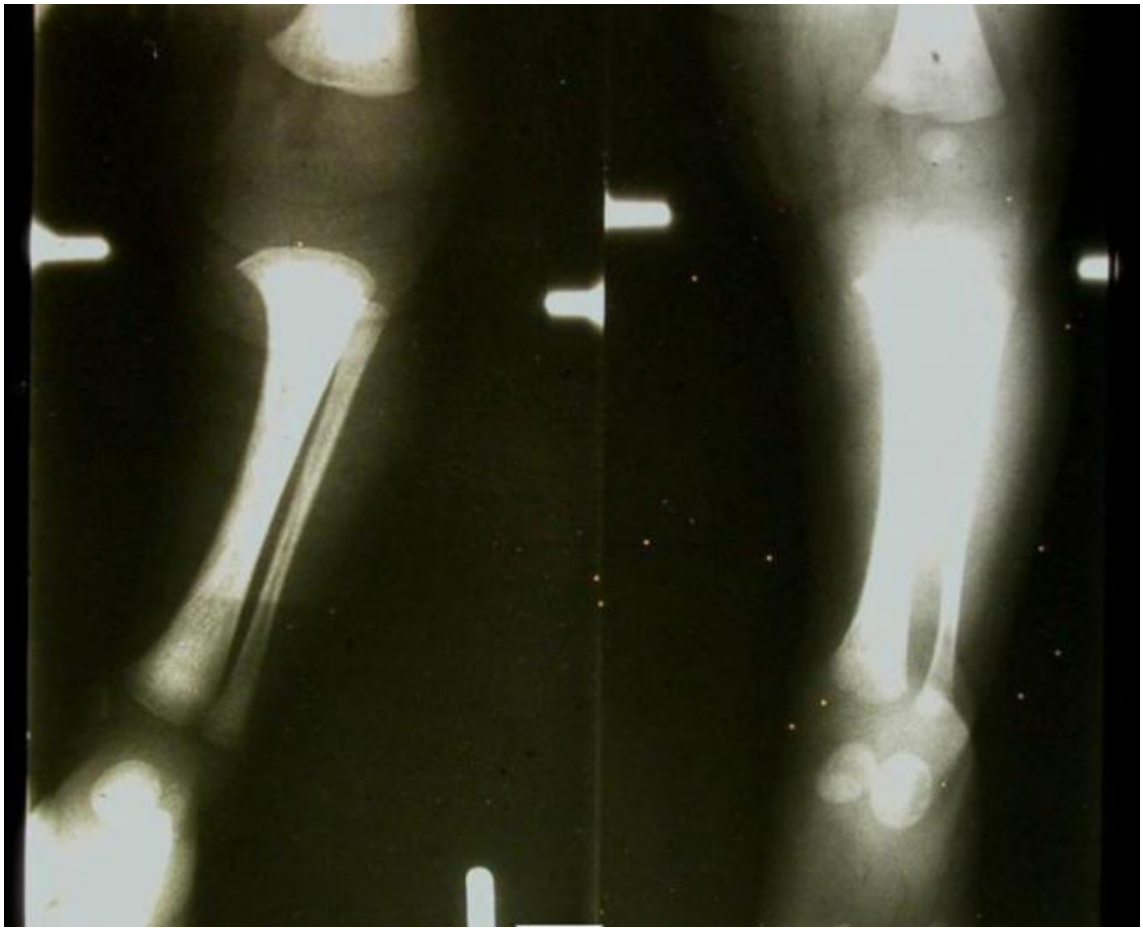


Figure 6: Radiograph of left lower extremity of two infants, (Left)Showing absence of distal femoral epiphysis, (Right) Distal femur showing presence of epiphysis in a normal child

These babies have a flat nasal bridge and the eyes could be mistaken for hypertelorism. The mouth may remain open, revealing the macroglossia. Further evaluation reveals a protuberant abdomen with an umbilical hernia. Skin appears cold

and mottled suggesting circulatory compromise. Neurological features include hypotonia and delayed reflexes. Absent femoral epiphysis on the x-ray can be seen in about 54% of the cases[34].

THYROID DYSGENESIS

Thyroid dysgenesis is the most common developmental defect causing congenital hypothyroidism and currently accounts for half the cases. This condition is characterized by a defect in migration of the median anlage, usually resulting in an ectopic lingual thyroid. The affected individuals are generally left with these lingual thyroids as the only thyroid tissue in the body. However, histologically these ectopic thyroid tissue reveals a normal follicular architecture[35]. Therefore, the hypothyroidism that is associated with this condition varies in its severity and depends on the number of cells. In about 33% cases of thyroid dysgenesis, even the most sensitive thyroid scan is unable to pick up remnants of thyroid tissue(aplasia). In the remaining 66% of the cases, thyroid tissue is picked up anywhere between the tongue(lingual) to its normal position in the neck(hypoplasia)[36].

Thyroid dysgenesis is generally sporadic in its inheritance. However familial cases occur more frequently than by chance alone. Another important aspect is the discordancy that is almost always noticed amongst monozygotic twins. In order to reconcile with discrepant findings, a two hit hypothesis, combining genetic susceptibility and early post-zygotic mutations have been proposed[37].

THYROID HORMONE DYSHORMONOGENESIS

Thyroid hormone dyshormonogenesis occurs when there is a defect in one or more of the steps leading to thyroid hormone formation and is its seen in about 15-20% cases of congenital hypothyroidism[38]. In these babies a the decreased thyroid hormone formation leads to an increased production of TSH hormone by the anterior pituitary through its negative feedback mechanism. This leads to an increase in the size of the thyroid gland and consequently many of these babies may either be born with a goiter or may develop it later, especially when diagnosis is delayed and treatment with levothyroxine is not initiated early[39].

Iodine and tyrosine form the major substrates in thyroid hormone synthesis. Iodine, a trace element could be one of the rate limiting factors in the synthesis of thyroxine. The process of thyroxine biosynthesis is initiated by the binding of TSH to the TSH receptor on the follicular cell and cAMP activation. Various processes are stimulated by cAMP and this includes cell membrane transport of iodine, synthesis of thyroglobulin, oxidation and organification of the trapped iodine, intracellular phagolysosome formation and hydrolysis of thyroglobulin in order to release iodotyrosines (monoiodotyrosine[MIT] & diiodotyrosine[DIT]) and iodothyronine(T4 &T3) residues, de-iodinisation of MIT & DIT by dehaologenase, leading to recycling of intracellular iodine and release of the T4 and T3 into circulation[37].

The cause thyroid dyshormonogenesisincude decrease in iodine trapping, defects in organification of trapped iodine, abnormalities in the thyroglobulin structure and defects in iodotyrosine de-iodination and recycling. From various molecular studies,

mutations in thyroperoxidase appears to be the most common etiology for thyroid dyshormogenesis. Identification of the specific disorder is however not of great importance as it has no bearing on the management[40].

SODIUM-IODINE SYMPORTER DEFECTS

Iodine transport across the cell membrane of the thyroid follicular cell from plasma to cytosol forms the first step in the synthesis of thyroid hormone. In normal individuals the iodine pump in the thyroid cell membrane is able to generate a thyroid/serum concentration gradient above 20 to 30. When the thyroid gland is stimulated by certain conditions such as low iodine diet, thyroid stimulation immunoglobulins, TSH, or drugs impairing the efficiency of thyroid hormone synthesis, this gradient can increase several hundredfold. The mapping of the sodium-iodine symporter gene(SCL5A5) located on chromosome 19 has allowed the detection of disease causing mutations in 31 babies with iodine transport defect as of 2006[37,41].

PENDRED SYNDROME

Pendred syndrome is a disorder that is transmitted my autosomal recessive inheritance and is characterized by goiter and congenital bilateral sensory neural hearing loss. Recent studies show that it is the cause of about 10% of the cases of congenital deafness[42]. The etiology of deafness in pendred syndrome remains controversial but CT scan of the temporal bone characteristically shows dilated semicircular canals(an abnormality that is also known as “Mondini’s cochlea”). The thyroid phenotype in this

condition is usually mild and its severity appears to depend on the nutritional iodine intake. Pendred syndrome is rarely picked up by newborn TSH screening[43]. Overtime, the affected children develop goiter and subtle hypothyroidism.

The gene responsible for this condition is identified to be SLC26A4 and is mapped to chromosome 7, and the protein pendrin is found to be a multifunctional anion exchanger. Pendrin is expressed mainly in the inner ear, thyroid and kidney. In thyroid, pendrin is localized to the thyrocyte apical membrane where it is involved in mediating influx of iodine[44].

THYROPEROXIDASE DEFECT

Organification of iodide requires two processes: oxidation of the iodide and iodination of the thyroglobulin bound tyrosine. The trapped iodide is first oxidized into an active intermediate, following which iodination of the thyroglobulin bound tyrosyl residues form iodothyrosines MIT and DIT. This iodination and coupling of tyrosyl is catalyzed by the thyroid peroxidase enzyme system in association with NADPH oxidases. The presentation of thyroid peroxidase deficiency is goitrous congenital hypothyroidism that is usually permanent with high serum levels of thyroglobulin and a scintiscanning showing high uptake[45].

The gene coding thyroid peroxidase is localized to chromosome 7 and the encoded glycoprotein is located on the apical membrane of the individual thyroid follicular cell.Though a variety of different mutations are described for this condition, attempts to identify a systematic genotype-phenotype correlation have been unsuccessful[46].

DEFECTS IN H2O2 GENERATION

Iodination of thyroglobulin catalyzed by thyroperoxidase and subsequent oxidative coupling of the iodinated tyrosyl residues to a protein bound iodothyronine is one of the main reaction in thyroid hormone synthesis[47]. When the supple of iodine is sufficient, availability of hydrogen peroxidase (H2O2) is the rate limiting factor for both these steps. DUOX2 (NADPH oxidase complex of dual oxidase 2) and DUOXA2 (DUOX maturation factor 2) are the primary enzymes required for feeding the H2O2 to thyroid peroxidase at the apical plasma membrane. The biological effects of thyrotropin receptor is mostly mediated by Gs/adenyl cyclase / cAMP pathway and the Gq/phospholipase C beta cascade is responsible for inducing H2O2 generation via its synergestic effect causing increased calcium and protein kinase C activation on the DUOX2 and DUOXA2. Defects in the generation of the thyroidal H202 and loss of function mutations in DUOXA2 and DUOX2 have been identified in babies with congenital hypothyroidism[48].

WHAT IS THE NEED FOR A NEWBORN SCREENING PROGRAMME FOR CONGENITAL HYPOTHYROIDISM?

As described above, thyroid hormones are absolutely essential for various metabolic functions as well myelination of the developing brain. Deficiency of thyroid hormones during critical periods of development results in severe and irreversible developmental retardation. congenital hypothyroidism is the most preventable cause of mental retardation in children.

Unfortunately the clinical symptoms of congenital hypothyroidism may take several weeks to months after birth to manifest. Therefore in the pre-newborn screening era, < 10% of children with congenital hypothyroidism were diagnosed by 1 month of age[49]. This was reported by Jacobsen et al in 1981. Between 1970-1975, only 10% of the Danish babies with congenital hypothyroidism were diagnosed in the first month of life. By the 3rd month, 35% of them were diagnosed and even at one year of age, only 70% with CH were diagnosed. Approximately 30% children remained undiagnosed till 3-4^{h} years of age.[49]. In the 21st century, similar findings were reported from a single hospital in New Delhi where between 1997-2010,260 children with CH presented of which 34 presentedat > 5 yrs of age[50]

Delayed diagnosis and initiation of treatment causes irreversible neurodevelopmental sequelae in children, in fact several IQpoints are lost with every week delay in treatment(ref Fig7 below). Therefore it is imperative that CH is identified as early as possible after birth and treatment initiated. The only way to identify CH early is by instituting NBS for all babies. With minimal cost of diagnosis, treatment and excellent outcome, NBS for CH is one of the most cost-effective screening programmes.

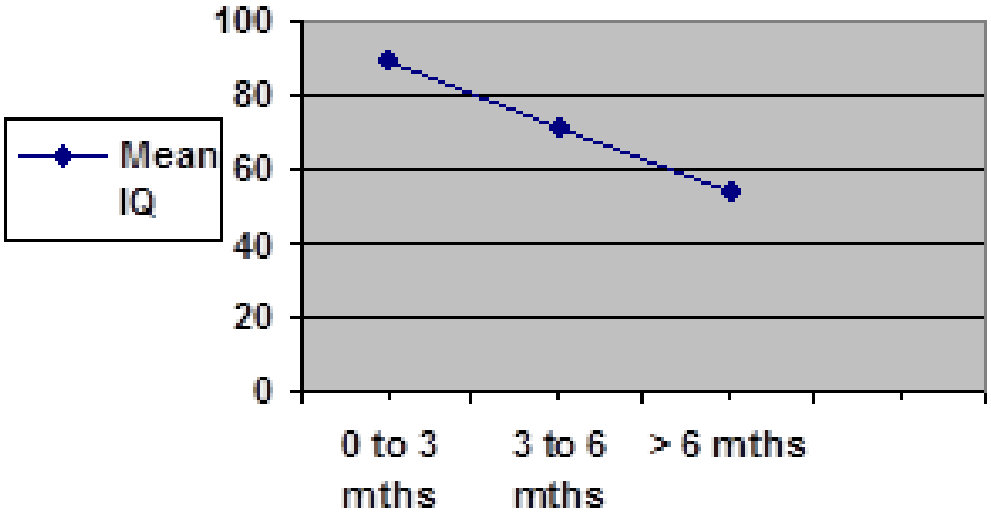


Figure7: Graph showing the relation between loss of IQ points and the delay in starting thyroxine in congenital hypothyroidism.(Adapted fromLa Franchi SH, Austin J. How should we be treating children with congenital hypothyroidism JPEM 2007)

EVOLUTION OF NEWBORN SCREENING FOR CONGENITAL HYPOTHYROIDISM

The concept of NBS for phenylketonuria using dried blood spot was first introduced by Prof.Guthrie in 1960[ref]. Screening for congenital hypothyroidism was added on in 1965. It was introduced in Quebec, Canada in the 1970s. In the mid 1970's it was observed that incidence ofcongenital hypothyroidism was twice as that of phenylketonuria.[51]Over the next two decades NBS for CH was adopted by most the developed nations of the world.In all these countries national newborn screening is entirely funded by the government. In recent years many of the developing

countries also have initiated screening programmes which combine several metabolic and endocrine disorders.

In India, NBS for CH was first introduced at Wadia Hospital, Mumbai in 1982 as a pilot programme[52].In 2007ICMR initiated a pilot study to screen CH and CAH at 5 regional centres to represent the North, West, South, Central and Eastern India to screen a target of 100,000 babies[53].Following this initiative, Goa was the first state to initiate state level government based comprehensive NBS "Heel to Heal" in 2008. This was followed by government sponsored screening programs at selected districts of Chandigarh, Gujarat, Kerala,Tamil Nadu and Delhi.In the last decade, NBS was started by few hospitals in several parts of India both in the private sector as well as at government facilities.

Christian Medical College, Vellore is a tertiary hospital with ~2800 inpatients and ~7000 outpatients every day. There are ~40-50 deliveries every day. An ongoing NBS for CH was introduced in July 2001 and has been ongoing since then. Having completed screening 1,65,637 babies, this is the largest existing cohort in India from a single institution.

PRIMARY TSH VERSUS PRIMARY T4 WITH TSH BACK-UP SAMPLING

In most countries such as USA, Canada, Mexico and Europe, primary TSH is done with back up T4 in those patients with an elevated primary TSH value. However, using this approach patients with thyroid binding globulin deficiency, hypothyroxinemia and central hypothyroidism will be missed, as in these babies the

elevation in the TSH is delayed. The other issue with this approach is that, with most centres discharging the mothers and babies before 48 hours of life, collecting the primary TSH sample in the first 48 hours can lead to a falsely high TSH value which is due to the normal physiological TSH surge and not due to any thyroid disorder. With the recent improvement in the TSH assay techniques using nonradioactive assays, we are currently able to more sensitively separate abnormal and normal TSH concentration. This has hence been instrumental in many countries looking to the primary TSH technique for their screening approach[54].

The second approach is a primary filter paper sample of T4 with a back up TSH done in those babies who had a low primary T4 concentration. This approach is useful in diagnosing babies with CH who have an initial low T4 with elevated TSH. This approach also helps in diagnosing infants with central hypothyroidism, thyroglobulin deficiency and hyperthyroxinemia. The disadvantage of this approach is that it will miss those cases of CH in which there is an initial normal T4 with delayed elevation of TSH[54].Another important consideration for the primary T4 approach is the more cost it entails.

Primary TSH screen is more sensitive and specific for the diagnosis of primary CH. Overall, primary TSH-based CH screening is more practical and cost-effective and is followed in most parts of the world.

CORD BLOOD VERSUS POSTNATAL SAMPLING- WHICH IS MORE FEASIBLE IN INDIA?

It is well known that at birth the mean CBTSH is ~10 mU/L[55]followed by a surge within 30 minutes which peaks by 24 hours of age to 60-70mU/L . This gradually declines to normal range by 72 hours. Because of this physiological pattern, TSH sampling for newborn screening should be collected either from the cord blood (before the surge occurs) or after 72 hours after birth to minimize high false positivity and recall rates.

In India, 70- 80 % mothers are discharged within 48 hours after delivery. If NBS is based on sampling > 72 hours of age, several children may miss creening because of early post-natal discharge. Post-discharge sampling relies on parents to bring back to hospital a “healthy normal” newborn baby for “blood” test. Unless there are facilities for home visits by health workers to collect dried blood spot samples, this may result in high rate of “missed screening”. It is important to remember that the major disadvantages with CB screening are that simultaneous screening for other IEM not possible, only institutional deliveries are covered, may miss out rare conditions like central hypothyroidism, primary CH with delayed rise and congenital TBG deficiency. With the rate of institutional deliveries steadily increasing in India[56], using CBTSH

TSH CUT-OFFS USED IN SCREENING PROGRAMMES

There is no consensus as to what is the most appropriate CBTSH to be used in screening programmes. Various centres across the country have been using different cut-off levels depending on their available data and experience. Devi et al considered

20µU/ml as an abnormal CBTSH in their study. However data from other studies use CBTSH values ranging from 9 µU/ml to 40 µU/ml as their set cut offs[57]. Considering that CBTSH is probably the most practical option of CH screening in India, it is important that a consensus cut off value for CBTSH is established so that centres all over the country could adopt it.

RESCREENING HIGH RISK INFANTS

International guidelines recommend that rescreening of all high risk newborns need to be done at 2 to 6 weeks of like. The high risk newborns include very low birth weight, premature and critically ill babies. The reason for the necessity of a delayed sample in premature babies is the relative prematurity of the hypothalamic-pituitary axis. Thus it is recommended that preterm babies have TSH samples done at 2, 6 and 10 weeks or when the baby is 1500 grams. The other reasons for a delayed rise in the TSH would be use of drugs such as steroids and dopamine. Though serial monitoring is recommended for these high risk infants, cost constraints and limited resources have made it difficult for most developing countries to establish it as a norm[58].

CORD BLOOD VS DAY 4 SAMPLING FOR NEWBORN SCREENING

In 1972 when newborn screening was first introduced in Quebec, 47000 newborns were screened over 3 years and 7 cases of congenital hypothyroidism was picked up. In view of the high frequency of false positives, delay in diagnosis and the increased cost involved, reference cut off values had to be devised.

In the year 1976, Walfish et al published in the Lancet that diagnosis of congenital hypothyroidism by cord blood TSH was a more sensitive and specific testwhen compared to T4 concentrations done on day 4 of life that had a high false positive rate. The author however concluded that T4 testing supplemented by TSH estimation would be the ideal screening modality. However doing both tests is not cost effective and is used only in few countries. Most centers in the USA and Canada prefer using the primary T4 testing approach while TSH testing is done mostly in Europe[59].

In 1982 the neonatal thyroid screening conference was held at Tokyo and they recommended that newborn screening programmed should be based on TSH concentrations in serum. It also recommended that this could be achieved by measuring TSH concentration obtained from dried blood spots on day 4 of life or by measuring T4 supplemented by TSH on the same filter paper only in those newborns who have a low T4 concentration.

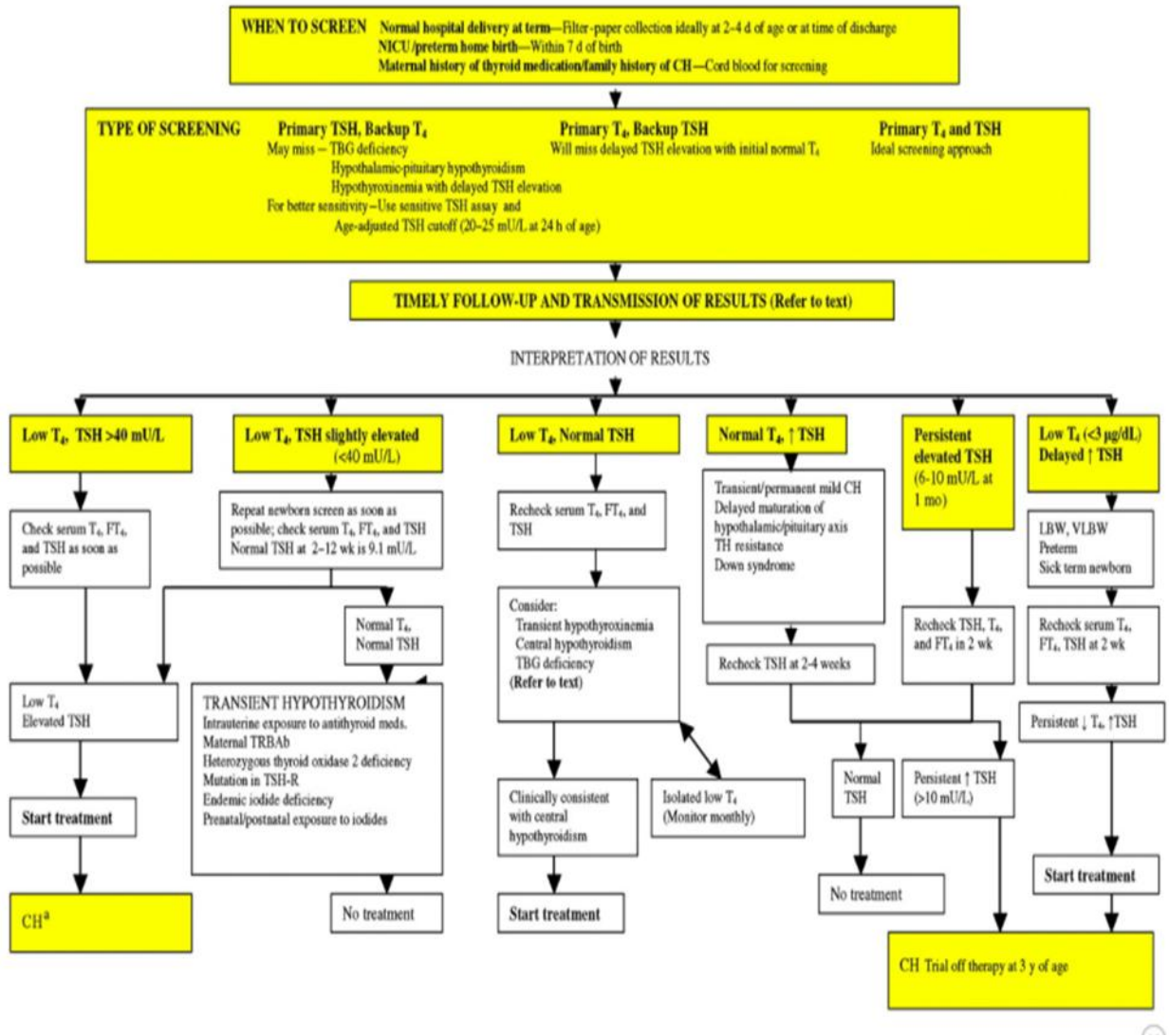


Figure 8. Approach to newborn screening and management of congenital hypothyroidism. Adapted from update of Newborn Screening and Therapy for Congenital Hypothyroidism | FROM THE AMERICAN ACADEMY OF PEDIATRICS [54]

CBTSH VS DAY 4 SAMPLING – PRACTICAL CONSIDERATIONS

The ideal method of newborn screening would be to do a filter paper sample for TSH and T4 on day 2 to 4 of life. It is preferable that blood samples are collected after day three of life as, even in normal babies there is a physiological surge in TSH levels

during labour which normalizes by day three of life. In most centers a primary TSH is done on day three of life with back up T4 being done if the TSH is abnormal. This method may however miss congenital hypothyroidism in three scenarios ie. TBG deficiency, hypothalamic-pitutary hypothyroidism and hypothyoxinemia with delayed elevation of TSH. Doing only a primary T4 sample would miss congenital hypothyroidism with delayed elevation of TSH with an initial normal T4[60].

In developing countries like India where follow up of patients after day three is difficult, one practical option would be to use cord blood TSH level as a screening tool. However this modality has certain drawbacks as the cord TSH level is affected by certain factors such as the maternal age, gestation, maternal and fetal iodine status with higher CBTSH levels having been reported in areas with iodine deficiency[61,62]. Withstanding these drawbacks, cord TSH has the exemplary advantage that it can be easily obtained in all newborns prior to discharge from hospital and a second visit is necessary only if the cord TSH is found to be abnormal. As per the CES data of 2009, institutional deliveries in India currently stand at 72.9% overall and is 85.6% in urban regions. This would mean that by introducing cord blood TSH as a national program we would be able to screen more than 3/4ths of the babies that are born in India each year. This we believe makes cord TSH the most pragmatic option for newborn screening for CH in India.

AIMS

To propose an ideal cord blood TSH cut-off level for mass screening for congenital hypothyroidism in the South Indian population. .

OBJECTIVES

1. To derive a sensitive, cost-effective cord blood TSH cut-off level for use in the mass newborn screening for congenital hypothyroidism in the South Indian population.

2. To propose this cord blood TSH level as a guideline to initiate national newborn thyroid screening program in India

METHODOLOGY

STUDY SETTING

Christian Medical College (CMC), Vellore is one of the first institutions in the country to initiate mass newborn thyroid screening programme CMC is a 2800 bedded tertiary care referral hospital located at Vellore in the southern state of Tamil Nadu.We have an ongoing screening programme and cord blood TSH (CBTSH) is being performed as standard of care for all babies born at our institution since July 2001.Babieswith cord blood above the cut-off level are recalled and sampled to confirm or rule out congenital hypothyroidism. In the initial 8 months of the screening programme, our CBTSH cut-off was 20 mIU/L and thereafter 25mIU/L. This study titled 'Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population' is an analysis of the data from our screening programme. We also prospectively recruited babies with cord blood TSH 20-24.99mIU/L during the study period. The sampling for the prospective wing was conducted in the Paediatric endocrinology project room located at the ISSCC building of the hospital or in the neonatal ICU.The departments involved in this study were the Paediatric Endocrinology division, Departments of Neonatology and Clinical Biochemistry.

STUDY DESIGN

There were two components to this study. The prospective component of the study involved recalling all babies born between January 2017- August 2017 with CBTSH between 20-24.9mIU/l, after 72 hours of age for repeat sampling of TSH/T4/FT4

levels. This part of the study also involved analyzing the initial 8 months of our screening programme data when the CBTSH cut-off was 20 mIU/L.

The second component involved analysis of data of all babies from the screening programme from July 2001 till June 2017 who had cord TSH between 25mIU/L to 30mIU/L. The correlation between CBTSH for every value between 25-30mIU/L and repeat TSH/T4 and FT4 levels wereanalyzed and a positive predictive value for each cord blood TSH level was derived.

The prospective data obtained wasanalysedto evaluate whether children with CBTSH between 20mIU/L- 24.9mIU/L are being diagnosed with CH. The screening data analysis was used to derive an ideal CBTSH cut-off for mass newborn thyroid screening with high sensitivity and low false positivity. The inclusion and exclusion criteria were:

INCLUSION CRITERIA :

1. Prospective arm: All babies born > 26 weeks of gestation born in CMC Hospital during the study period with CBTSH levels between 20-24.9 mIU/L.

2. Screening data analysis involves data from all the participants of newborn thyroid screening programme in CMC between June 2001- July 2017 with CBTSH levels between 25-30mIU/L.

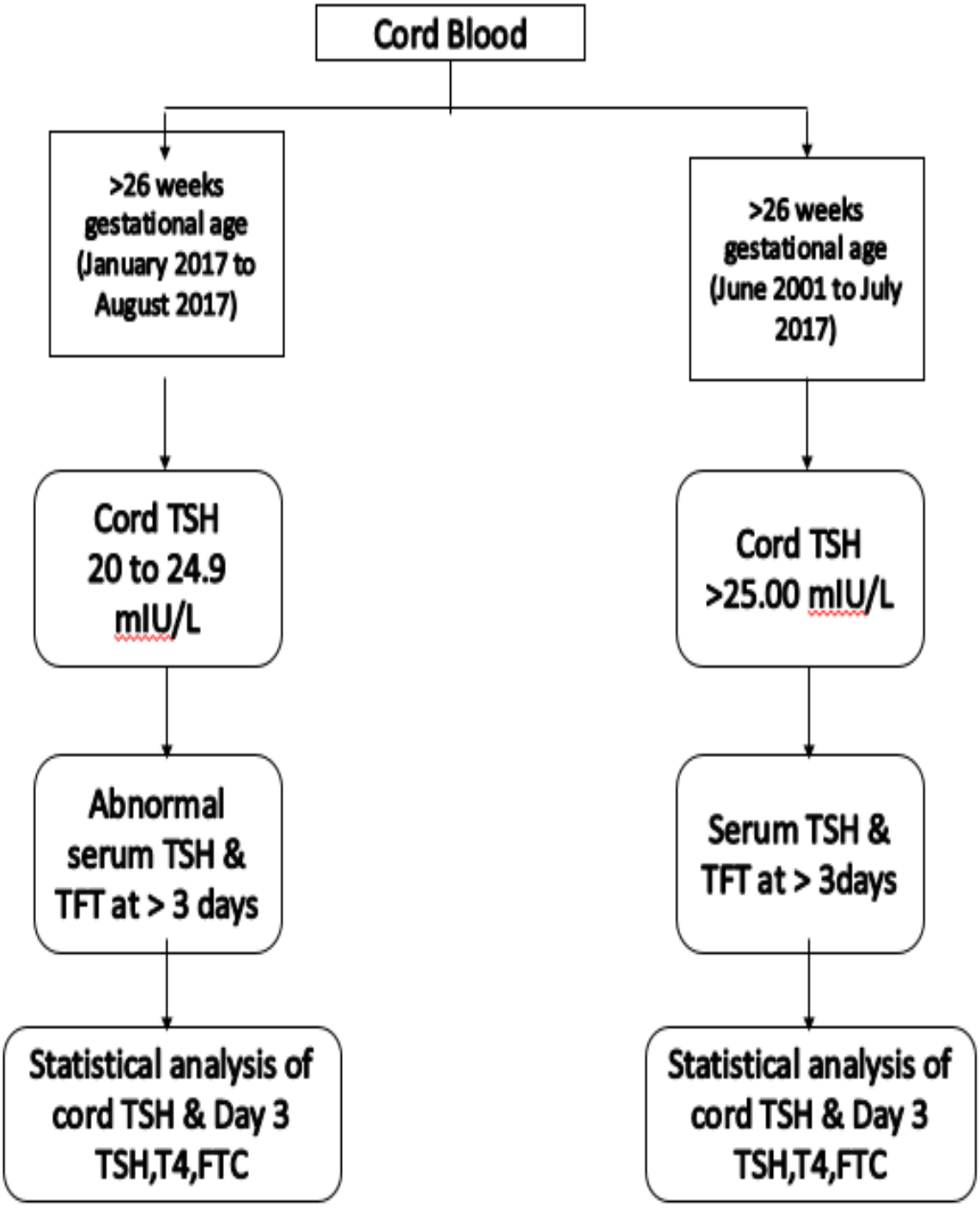
EXCLUSION CRITERIA:

1. All babies who were critically ill in the first 72 hours requiring NICU admission.

2. Babies in whom screening was missed at birth

3. In the prospective arm, babies whose parents did not consent to return for sampling after 72 hours of life.

DIAGNOSTIC ALGORITHM OF THE STUDY



DESCRIPTION OF VARIABLES AND OUTCOMES

The variables studies in this study are cord TSH, collected at the time of delivery and TSH, T4 and FTC done after day three of life in those babies who had a high cord TSH value. When venous blood sample is used to measure TSH it is expressed as serum units. Assays on dried blood spot samples are expressed as whole blood units. Whole blood units may be converted to serum units by multiplying by 2.2. In this study TSH is uniformly mentioned as serum units.

The primary outcome measured here is a diagnosis of congenital hypothyroidism made in those babies who were found to have high cord blood TSH concentrations and then went on to have an abnormal TSH, T4 and FTC level when it was repeated after day three of life.

DATA SOURCE MEASUREMENT AND MANAGEMENT

All newborns born in CMC > 26 weeks of gestation have their cord blood TSH screening done as routine standard of care. During the study period, those with CBTSH between 20-25 mIu/l wererecruited into the study after parental consent and recalled for repeat sampling beyond 72 hours of age. Cord blood TSH and repeat venous samples of TSH, T4 and FT4 were analysed at the biochemistry lab using the CENTAUR automated chemi-luminiscence imunoassay.

All qualitative and quantitative variables in the prospective component of the study was entered into a clinical proforma. The clinical proforma used in this study is attached as Annexure I.

The data in the retrospective component of the study was obtained from the Paediatric endocrine database maintained in the unit since the inception of the newborn screening program in the year 2001.

BIAS

All babies who had CBTSH levels between 20-24.9 mIU/L during the study period were invited to participate . Only those children who were unwell needing NICU admission and those whose parents refusedparticipationwere excluded thereby minimising the potential selection bias.

SAMPLE SIZE CALCULATION

In the retrospective component of the study all newborn babies born between July 2001 to June 2017 for whom cord blood TSH was done were included in the study.

In the prospective component of the study, sample size calculation was done on the basis of our own screening data. . In the period between July 2001 and December 2015 cord blood TSH was performed on 1,44,636 babies and a diagnosis of congenital hypothyroidism was confirmed on 123 babies.This leads to a disease incidence of 0.8 per 1000, which was used as the statistical input for sample size calculation. The sample size was calculated using nMaster software version 2.0.

Formula

$$n = \frac{Z^2_{1-\alpha/2}\, p(1-p)}{d^2}$$

Where,

p : Expected proportion

d : Absolute precision

1- α/2 : Desired Confidence level

With proportion of 0.08 and absolute precision of 5% and for the 95% confidence interval, the sample size was calculated as 113.

STATISTICAL METHODS

For continuous data, the descriptive statistics n, mean, SD, median, IQR, minimum and maximum were used. For categorical data, the number of patients and percentage were calculated. Based on the normality of data, the parametric t test or non-parametric Mann Whitney test was used. Data was analysed by performing chi square and Fischer's test where applicable.

For each interval of cord TSH, the number and percentage were presented with histogram to examine the distribution of the data. The cut-off points of indices of cord TSH for predicting a diagnosis of congenital hypothyroidism was obtained by receiver operated characteristic (ROC) curve analysis. For each cut-off point, sensitivity, specificity, positive and negative predictive values for detecting congenital hypothyroidism were obtained.

Using verification bias, point estimates and confidence intervals were calculated.

P-values are reported as specified by the statistical software used, at least up to four decimal places. P-values less than 0.0001 are reported as provided by statistical software (e.g. '<0.0001'). All tests were two-sided at α=0.05 level of significance. All statistical analysis were done using SPSS software version 17.0 or later.

RESULTS

TSH level is uniformly mentioned as serum units.

CBTSH SCREENING JULY 2001-AUGUST 2017

Table 2: CBTSH in CMC Hospital (July 2001- August 2017)

Total deliveries	1,65,637
Total screened	1,64,163
Missed primary screening	1,264(0.76%)
Total recalled	5488 (3.31%)
Total resampled	4224 (2.54%)
Missed resampling	1264 (23%)
Recall rate	2.54%
Primary CH confirmed	123
Etiology of CH	Dyshormonogenesis 53(44.2%) Dysplasia 38(31.7%) Ectopia 26(21.6%)
Prevalence of CH	1: 1,346

MEAN CBTSH LEVELS (JANUARY 2005-JANUARY 2017)

The mean CBTSH levels over a 12 year period between Jan 2005 to January 2017 was analysed to assess whether there was any significant change in the CBTSH over the years. During this period CBTSH was performed on 1,28,159 babies. The mean TSH during each year is illustrated in Table 2. The mean TSH concentration over the 4 year period between 2007-2010 was 9.645 mIU/L and had decreased to 8.830 mIU/L between 2015-2017. The CBTSH was least in the year 2005 when it was 8.156±15.952 mIU/L and it reached a maximum value of 10.119±11.491 mIU/L in the year 2008.

A significant difference in the mean CBTSH concentration when compared to the previous year was noticed in the years 2006(p=<0.001), 2009(p=0.006), 2011(p= 0.002) and 2012(p=<0.001). Comparison of mean cord TSH concentration between the other years did not show any statistical difference. There was no definite pattern (consistent increase/ decrease) over 12 years.

Table 2. Mean cord TSH between 2005 -2017(n=1,28,159)

Year	N	Mean CBTSH(mIU/l)	Std. Deviation	p-value*
2005	5762	8.156	15.952	1.000
2006	7093	10.078	14.111	<0.001*
2007	8136	9.882	10.557	1.000
2008	8592	10.119	11.491	1.000
2009	9383	9.403	15.619	0.006*
2010	10350	9.285	13.392	1.000
2011	11535	9.982	10.362	0.002*
2012	12549	9.045	9.377	<0.001*
2013	13060	8.666	9.776	1.000
2014	13455	8.725	8.923	1.000
2015	13740	8.960	10.799	1.000
2016	13527	8.731	14.465	1.000
2017	959	8.356	6.679	1.000

*Significance of difference of means with the preceding year .

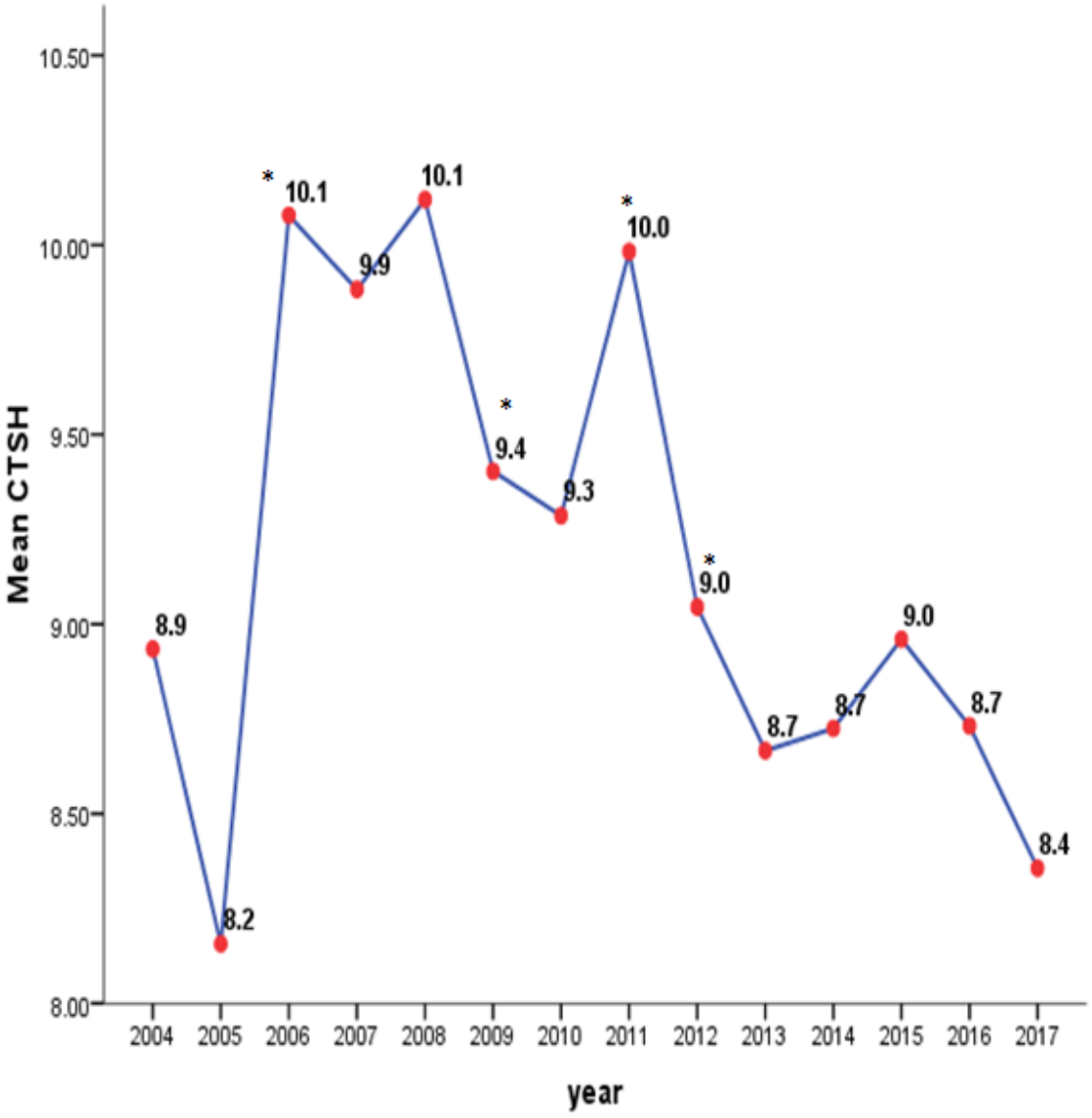
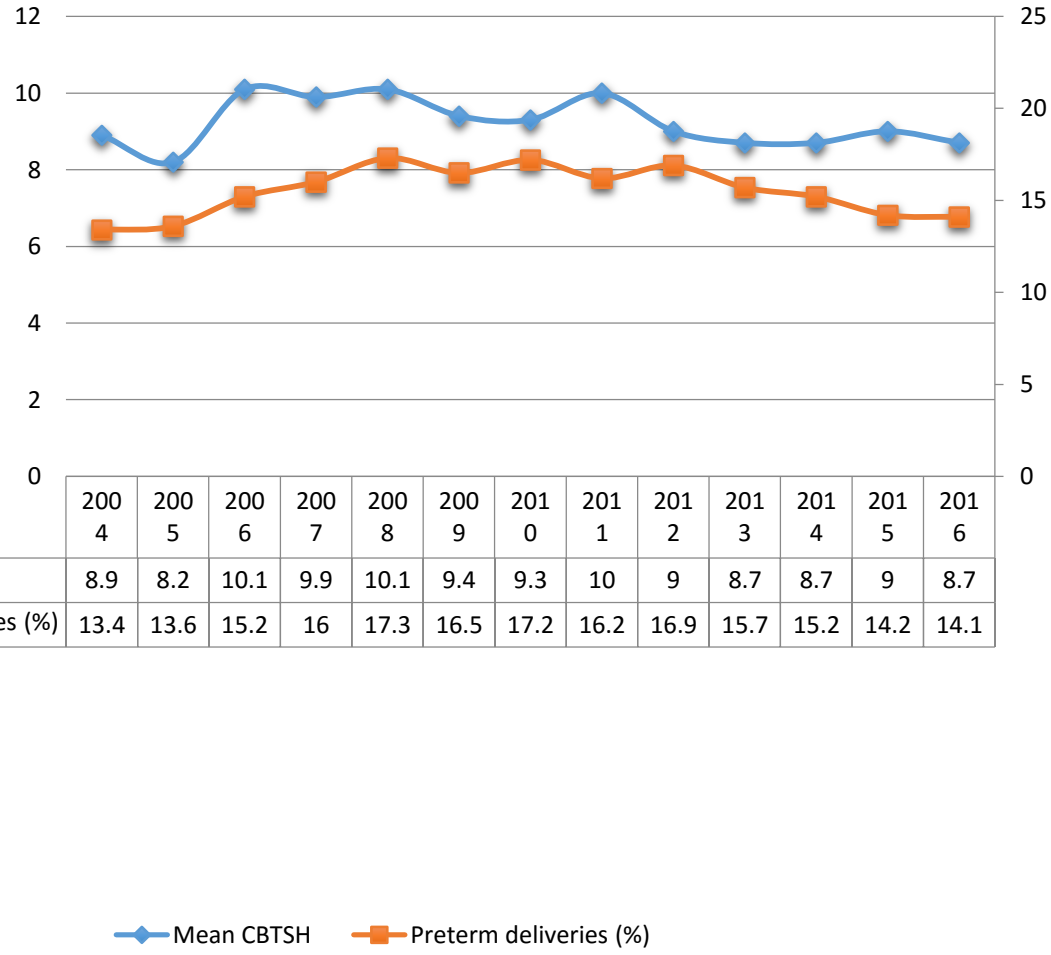


Figure 9. Graph showing CBTSH trend between years 2005-2017. Cord TSH measured in mIU/L is plotted along the "x" axis and the year of birth is plotted on the "y" axis.

CBTSH LEVELS AND PRETERM BIRTHS

The ***mean overall***CBTSH level for each year is shown against the preterm births for the corresponding year in Figure 2 below. 13-17% of the deliveries in our institution were preterm births.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Mean CBTSH	8.9	8.2	10.1	9.9	10.1	9.4	9.3	10	9	8.7	8.7	9	8.7
Preterm deliveries (%)	13.4	13.6	15.2	16	17.3	16.5	17.2	16.2	16.9	15.7	15.2	14.2	14.1

Figure 10. Graph showing the comparison of mean cord TSH with the number of preterm deliveries each year. In this graph, year of birth is plotted along the "x"axis, mean CBTSH along the left "y" axis and proportion of preterm deliveries along the right "y" axis.

CBTSH BETWEEN 20 – 24.99 mIU/L

Among the babies who had CBTSH concentration between 20-25mIU/L, two groups were included in the study: babies born between January 2017 to August 2017(prospective cohort) and those born between July 2001 to March 2002 (retrospective cohort).Overall during this period of 17 months 14,742 babies had CBTSH assays done. Of the 155 babies born with CBTSH 20-24.99mIU/L, 62 babies who returned for sampling were included in theprospective cohort. In the retrospective cohort, 133 of the 138 babies who were resampled were included. Thus a total of 195 babies were included in the analysis.

The mean duration at which repeat thyroid function tests was done was 10 ± 12.06 days in the prospective cohort and 6.84±8.53 days in the retrospective cohort. The baseline characteristics of babies with CBTSH 20 – 25mIU/L are shown in Table 3 below

Table 3. Mean cord TSH, repeat TSH, T4 and FTC among those with CBTSH between 20 - 25mIU/L (Results expressed as mean(SD)

N who had CBTSH assay over the study period: 14742

N with CBTSH 20-24.99 mIU/L : 293

N who had repeat sampling done: 195

N=195	Prospective cohort (Jan 2017 –Aug 2017)	Retrospective cohort (July 2001- March 2002)
CBTSH(mIU/L)	22.734± 1.918	22.267±1.485
TSH>72 hrs(mIU/L)	3.760± 2.783	4.140±3.382
T4(mcg/dl)	12.680±3.480	13.953±3.785
FT4(ng/dl)	1.841± 1.387	1.878±1.135
Confirmed CH	0	0

Birth weight	**<1500gm**	**1501-2500gm**	**2501-4000gm**	**>4000gm**
	0	7	55	0
Gestational age*	<37 weeks	37 -41 weeks		>41 weeks
	5	50		0
Mode of delivery*	LSCS	Normal delivery	Suction cup	Forceps
	5	37	7	7

Table 3.4. Baseline characteristics of babies with cord TSH 20 - 25mIU/L in the retrospective cohort (n=62)

*** Complete data was not available for 7 babies**

Table 3.4 shows the baseline characteristics of babies in the prospective cohort. Majority of the babies born with a cord TSH of 20-25mIU/L were of normal weight(2500-4000gm). 50 of the 62 babies were born at term and 37 of them were born by normal vaginal delivery.

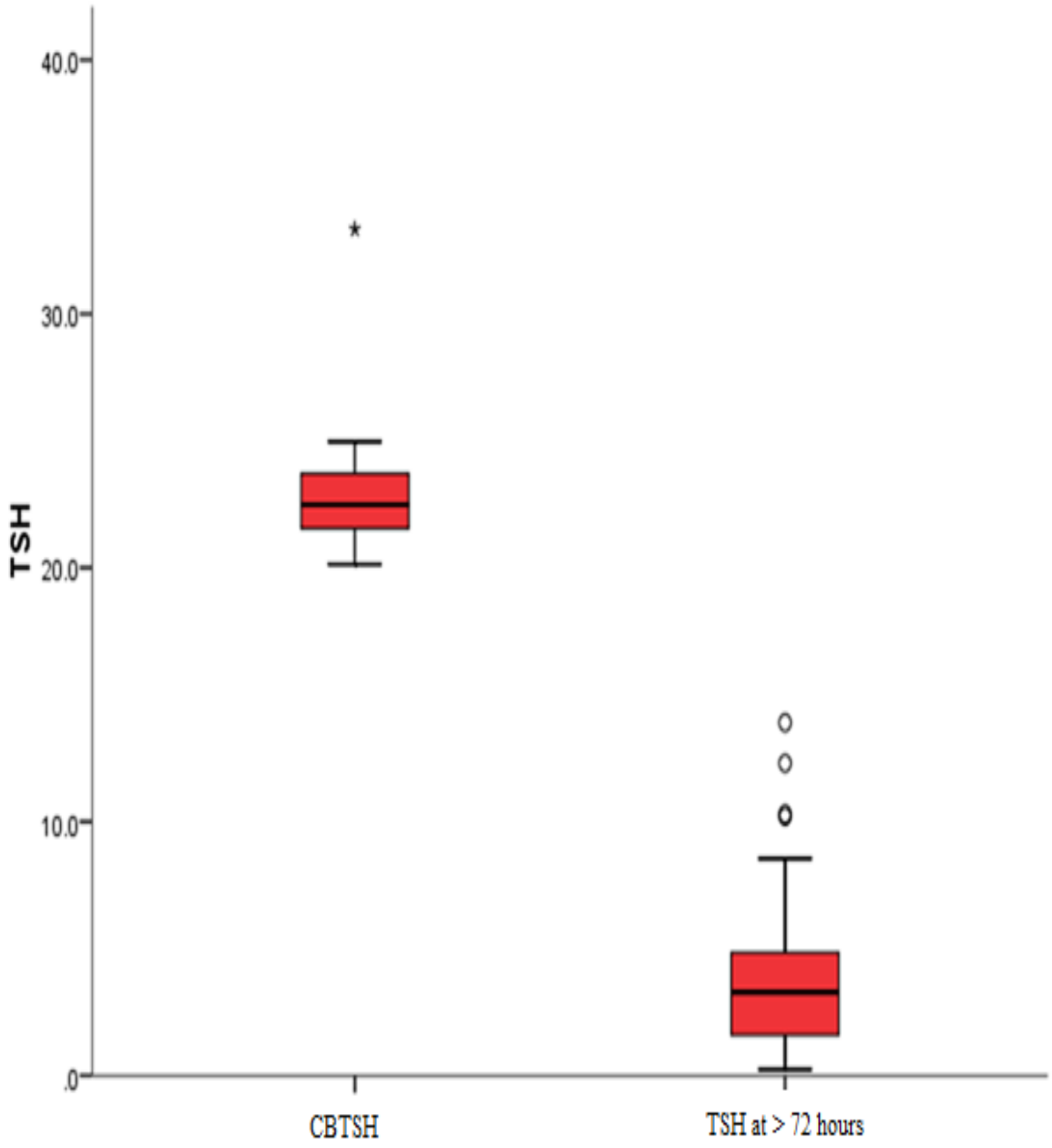


Figure 11:. Box and whisker plot showing the mean TSH concentrations at birth(CBTSH) and at >72 hours of age.

Table 5. Correlation between CBTSH and birth weight, gestational age and mode of delivery

Characteristic	Mean (S.D)	p-value
Birth weight (grams)	2979.07 (386.29)	0.254
Gestational age (weeks)	38.68 (1.33)	0.231
Mode of Delivery	**n (%)**	
LSCS	5 (7.9)	0.468
Normal vaginal delivery	37 (58.7)	
Forceps delivery	7 (11.1)	
Suction cup delivery	7 (11.1)	

The mean cord TSH concentration in the retrospective and prospective cohorts were22.734±1.92 mIU/L and 22.267±1.49 mIU/L respectively. There was no difference between the two mean CBTSH levels (p=0.065). The mean TSH concentration among all babies in the 20-25mIU/L group was 22.260mIU/L. The mean repeat TSH concentration in the prospective and retrospective cohorts were 3.760 and 4.140mIU/L respectively.

As shown in table 4 there was no correlation between the cord TSH and gestational age, birth weight or mode of delivery in those babies who had a CBTSH between 20.0-24.9mIU/L. There were no confirmed cases of congenital hypothyroidism in this group.

Between July 2001 to March 2002, 5209 babies were born, of which 138 had CBTSH between 20-25mIU/L. Between the period of January 2017 to August 2017, 9533

babies born, of which 155 babies had a CBTSH OF 20-25mIU/L. Overall ~2% babies wereborn with CBTSH 20-25mIU/L

CBTSH BETWEEN 25 – 30 mIU/L(JULY 2001-AUGUST 2017)

Over the period between July 2001 and August 2017, CBTSH was performed on a total of 1,65,637 babies. Of these, 5488 babies had CBTSH between 25-30mIU/L(3.31%). The ratio of males:females was 1.39:1.Their baseline characteristics are shown below in Table 6

Table 6. Baseline characteristics of babies with CBTSH 25- 30 mIU/L

(values in parenthesis indicate percentage unless specified otherwise)

Total screened=1,65,637 **CBTSH >25mIU/L= 5488**	
Characteristic	Value
Birth weight (mean±SD)	2877.49 ± 639.43
Gestational age (mean±SD)	38.5 ± 4.59
Male	3196 (58.2)
Mode of Delivery	
LSCS	597 (10.9)
Normal Delivery	3390 (61.7)
Forceps	534 (10.6)
Suction cup	525 (10.4)
Breech	9 (0.2)

Of the 5488 babies who were recalled, 4224 babies returned for resampling. (76.96%). The mean age at which the blood tests were repeated was 7.16 ±19.56 days. The mean repeat TSH (> 72 hours of age) was 10.13 ± 56.89 mIU/L.

Table 7. Mean cord TSH , repeat TSH and TFT in the >25mIU/L group

Characteristic	Value
CB TSH (mIU/L)	39.71 ± 50.37
Age of resampling (days)	7.16 ±19.56
Repeat TSH (mIU/L)	10.13 ± 56.89
Total T4(mcg/dl)	13.73 ± 4.76
FT4(ng/dl)	1.68 ± 0.76

Table 8. Mean CBTSH in those babies in whom CH was excluded and those with diagnosed CH

	N	Mean±SD	Range
Cord TSH in those babies in whom CH was excluded	4104	35.32±24.62	25.00 - 247.00 mIU/L
Cord TSH in those babies diagnosed with CH	120	240.04±218.78	25.67 – 1073.00mIU/L

Table 7 shows CBTSH in those babies in whom CH was excluded and those with diagnosed CH. Mean CBTSH in those in whom CH was excluded was 35.32±24.62 mIU/L. However mean CBTSH in those with CH was 240.04±218.78 mIU/L.

Three babies with CBTSH 149.30,159.00 and 201.00 mIU/L shown as CH excluded in the Table above were severely asphyxiated and expired/ discharged against medical advice

Table 9. Mean CBTSH trend in >25mIU/L group

Time Period	N	Mean ± SD	Range	No diagnosed to have Congenital hypothyroidism(%)
2001 – 2004	751	35.69 ± 27.09	25.00 – 655.00	18(0.04)
2005 – 2008	1258	41.29 ± 57.91	25.03 – 1070.00	25(0.02)
2009 – 2012	1647	40.91 ± 58.02	35.98 ± 15.92	31(0.02)
2013 – 2017	1832	39.22 ± 44.33	25.01 – 750.00	46(0.03)

The mean cord TSH in the >25mIU/L group was 39.71 ± 50.37. The mean cord TSH trend in this group is shown in table.5; the lowest was 35.69 ± 27.09 mIU/L(2001-2004) and highest was 41.29 ± 57.91mIU/L(2005-2008).

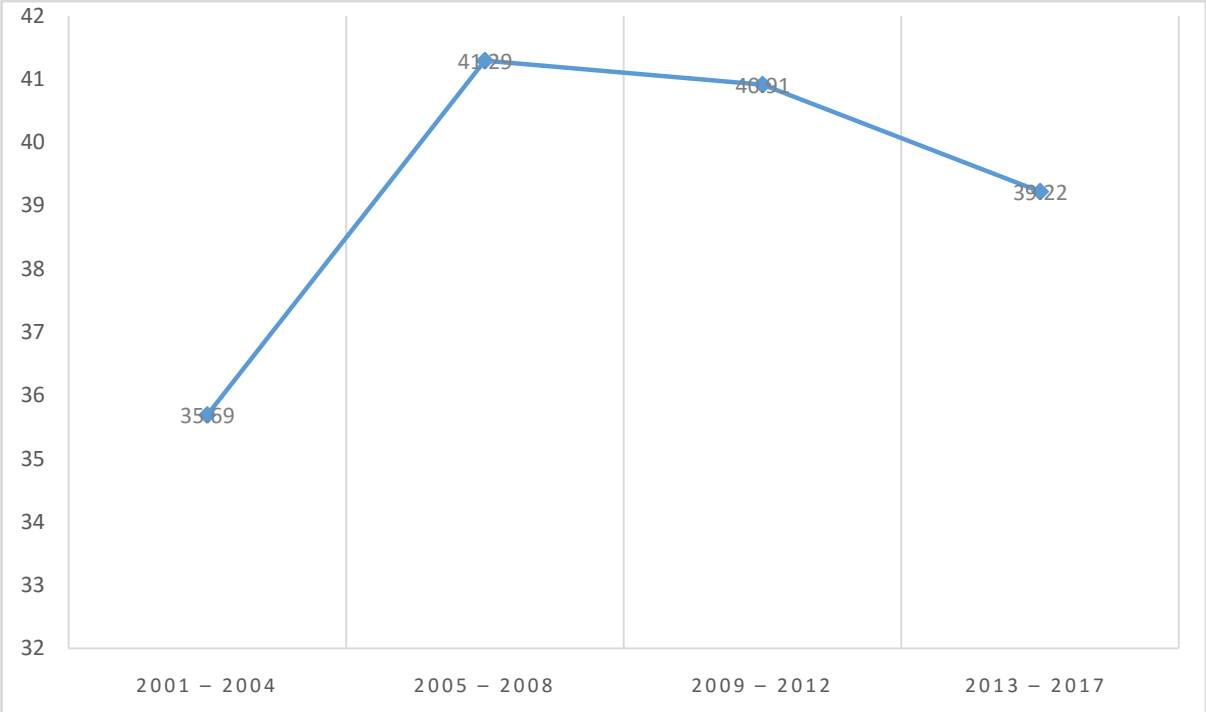


Figure 12. Graph showing the cord TSH trend in the >25mIU/L

Table 10. Comparison of cord TSH with gestational age, birth weight and mode of delivery by means of a Correlation coefficient.

Characteristic	Correlation coefficient (corr)	p-value
Cord TSH and Gestational age	0.004	0.779
Cord TSH and Birth weight	0.020	0.134
Cord TSH and Mode of delivery	0.062	<0.001

Table 7 shows the comparison of association of cord TSH with gestational age, birth weight and mode of delivery by means of a Correlation coefficient. Only the mode of delivery(instrumental delivery, forceps and suction cup) had a significant correlation with the cord TSH levels; instrumental delivery was associated with a higher level of CBTSH($p<0.001$).

BABIES WITH CBTSH>25 MIU/L RECALLED, BUT DID NOT COME FOR RESAMPLING

Table 11. Mean cord TSH among those babies who did not return for repeat testing

Time Period	N(%)	Mean ± SD	Range
2001 – 2004	109(14.5)	35.21 ± 11.97	25.10 – 98.00
2005 – 2008	400(27.5)	40.08 ± 58.25	25.10 – 149.30
2009 – 2012	353(21.4)	35.98 ± 15.92	25.08 – 159.00
2013 – 2017	395(21.6)	33.70 ± 13.48	25.01 – 201.00

Of the total of 5488 babies recalled for testing, repeat blood tests were performed in 4224 babies who returned for sampling.Figure 7 shows the year wise trend of number of babies who did not return for repeat testing and their mean cord TSH concentration.

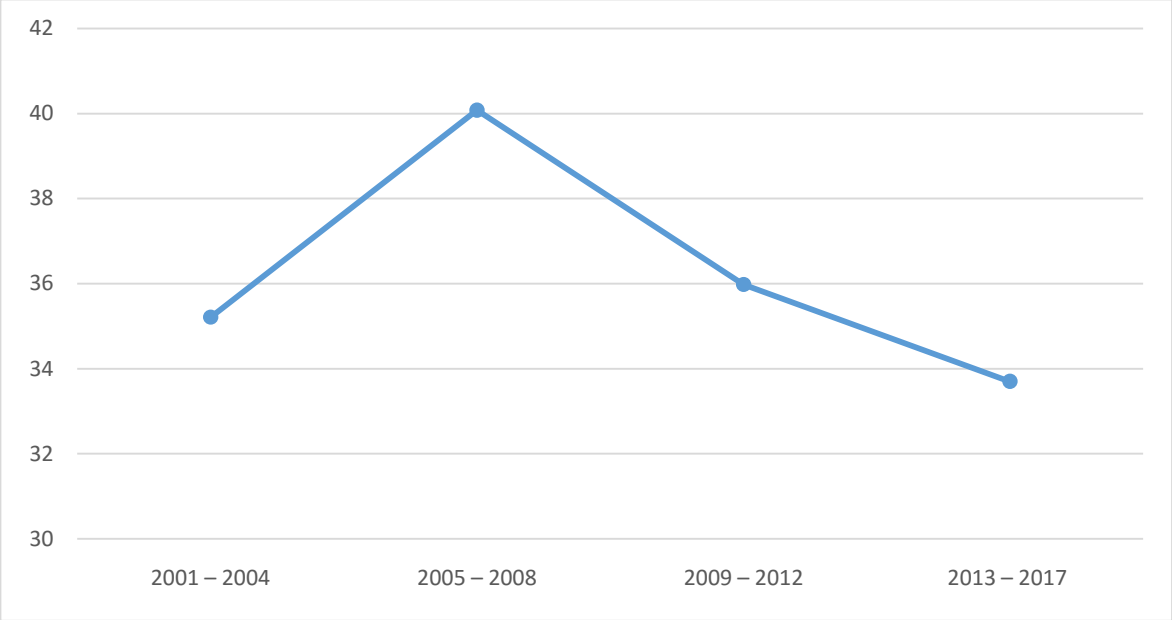


Figure 13. Mean Cord TSH trend amongst those babies who did not return for repeat testing

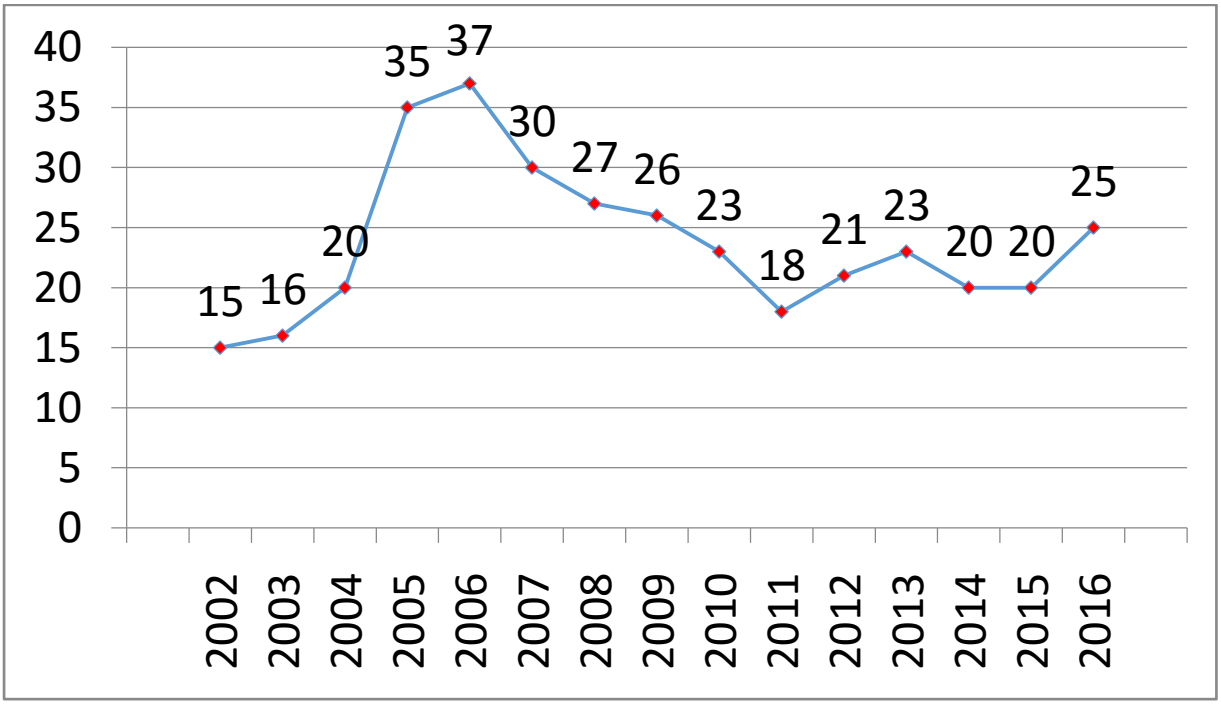


Figure 14. Proportion of babies who did not return for confirmatory sampling . In this graph, year of birth is plotted on the "x" axis and proportions are plotted on the "y" axis

In the period between 2001-2004, only 109 babies missed confirmatory sampling.However the years after that saw a significant increase in the babies in whom confirmatory sampling could not be performed.

BABIES CONFIRMED TO HAVE CONGENITAL HYPOTHYROIDISM

Of the 1,66637 babies screened, diagnosis of congenital hypothyroidism was confirmed in 123 babies. 120 of these babies had nuclear imaging done prior to treatment initiation. Of these 53 babies had dyshormonogenesis(enlarged/normal gland), 38 had thyroid dysgenesis(agenesis/hypoplasia) and 26 had ectopia.

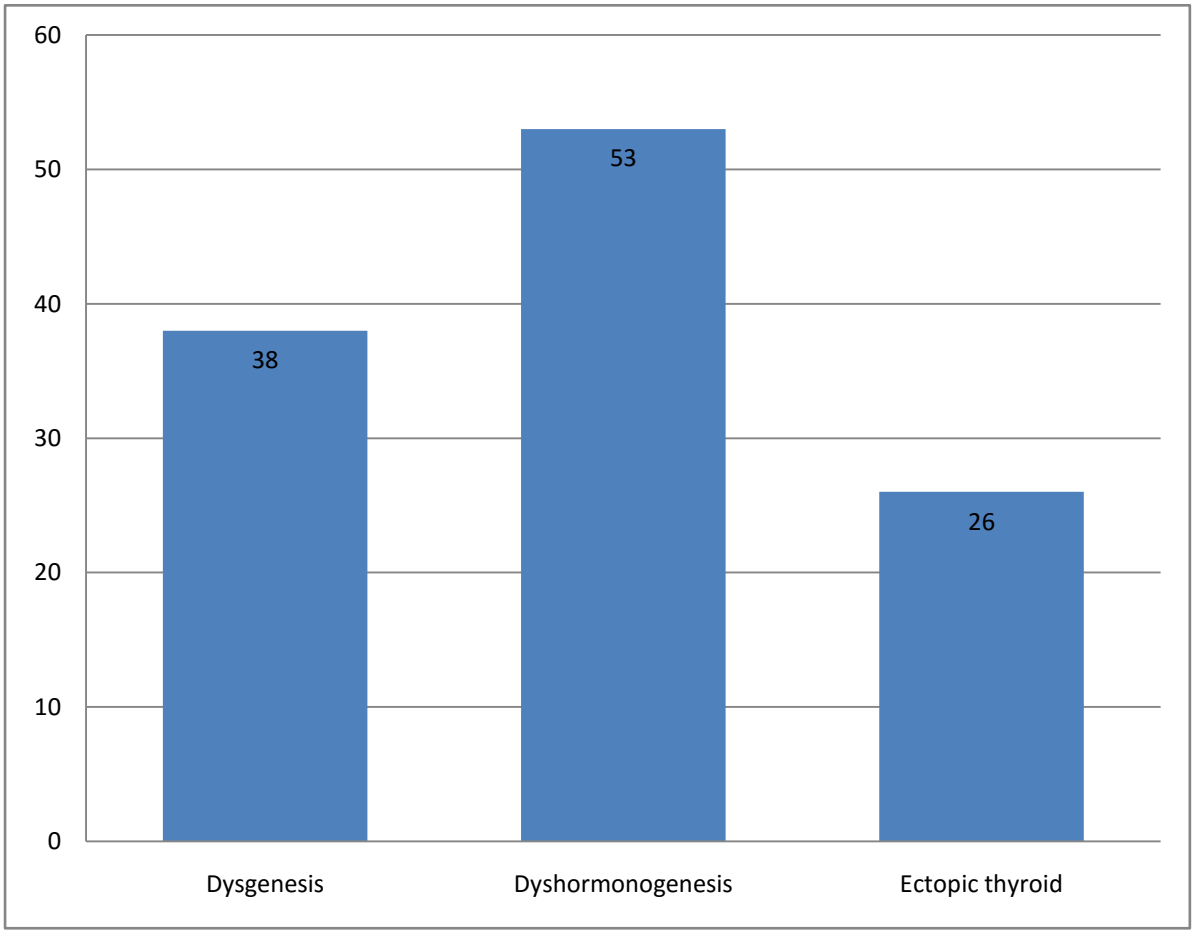


Figure 15. Aetiologicalclassification of confirmed congenital hypothyroidism

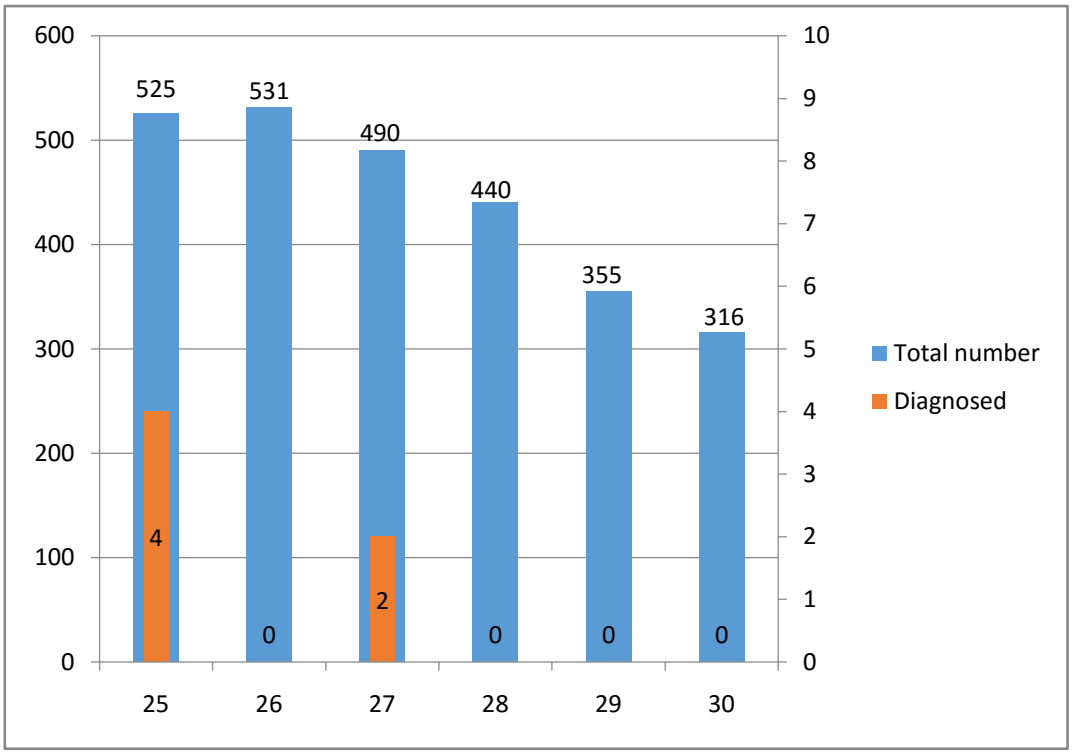
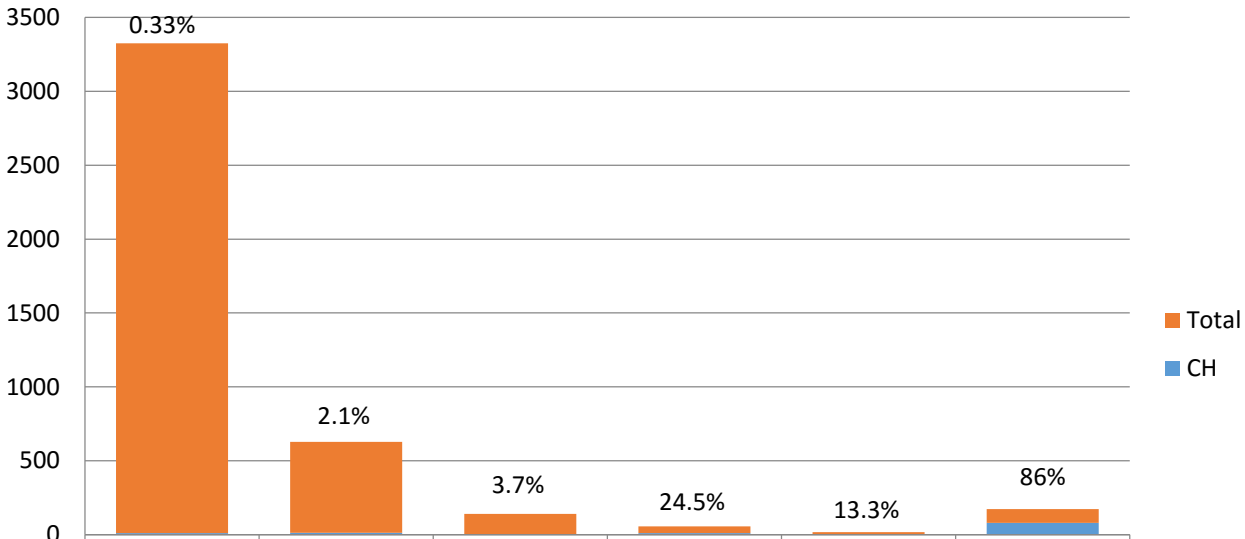


Figure 16. Graph showing confirmed CH in those recalled for confirmatory sampling. In the graph the CBTSH interval is plotted alone the 'x' axis, the number of subjects in each CBTSH interval is plotted along the left "y" axis and the confirmed CH along the right "y" axis.

Table 12.Association between CBTSH level and aetiology of CH

Outcome	Frequency - n	Cord TSH (mean±SD)	Range	p value
Dysgenesis	38	300.33 ± 254.74	27.5-750	<0.000
Dyshormonogenesis	53	222.39 ± 214.76	25.29-731	<0.000
Ectopic Thyroid	26	198.26 ± 165.29	31.59-750	<0.000

Table 9 illustrates the mean CBTSH concentration in each aetiology of congenital hypothyroidism. Thyroid dysgenesis was associated with the highest CBTSH 300.33 ± 254.74 mIU/L followed by dyshormonogenesis and ectopia.There was significant



	25 - 40	41 - 55	56 - 70	71 - 85	86 - 100	>100
Total	3313	615	136	45	15	93
CH	11	13	5	11	2	80

difference in the mean CBTSH levels of each of these 3 aetiologies.

Figure 17. Representation of number of babies diagnosed with CH in each interval of CBTSH value

Figure 10 represents the number of babies diagnosed with CH in different intervals of CBTSH. Eleven of the babies in the 25-40mIU/L group were diagnosed with CH. Only 2 of the 15 babies in the 86-100mIU/L grouphad CH, while 80 out of the 93

babies in the >100mIU/L had a diagnosis of CH. It is important to note here that 14 % of babies who had a CBTSH >100mIU/L did not have CH.

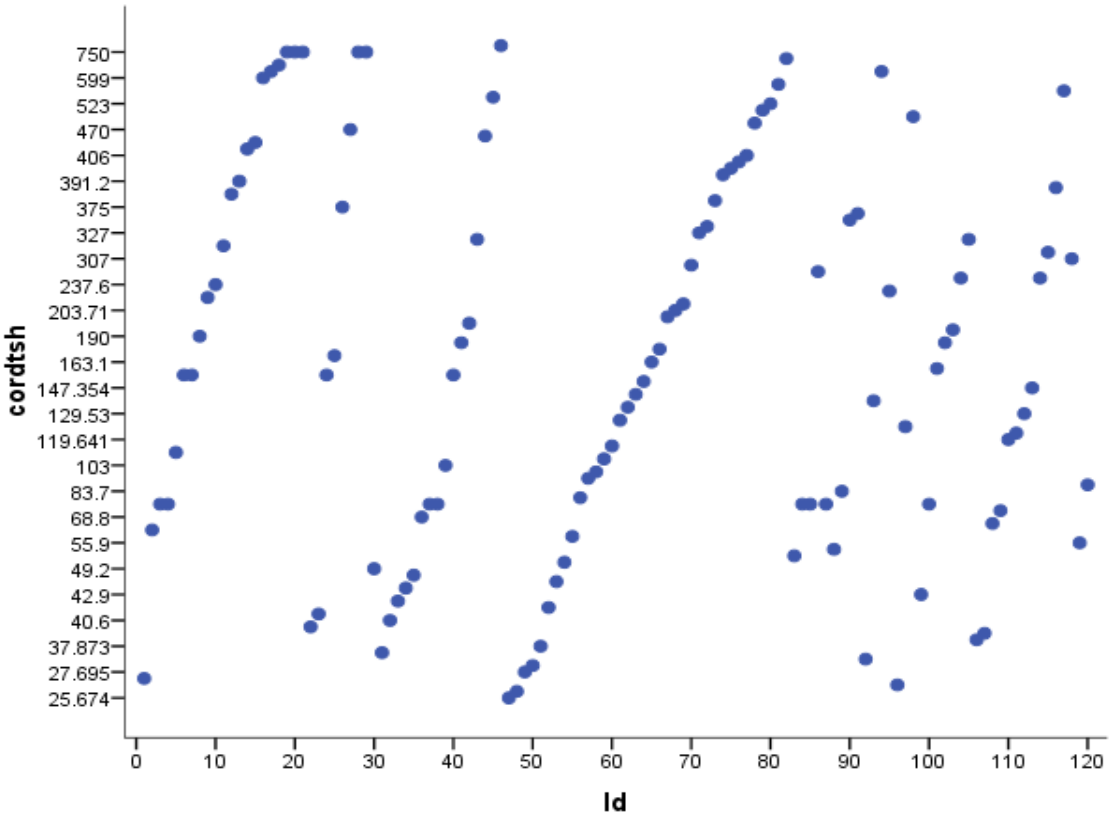


Figure 18. Independence plot with patient ID plotted on the 'x' axis and CBTSH on the 'y' axis

In the above scatter plot where CBTSH of all babies with a diagnosis CH are plotted, there is a clear pattern in the CBTSH seen above a concentration of 45mIU/L.

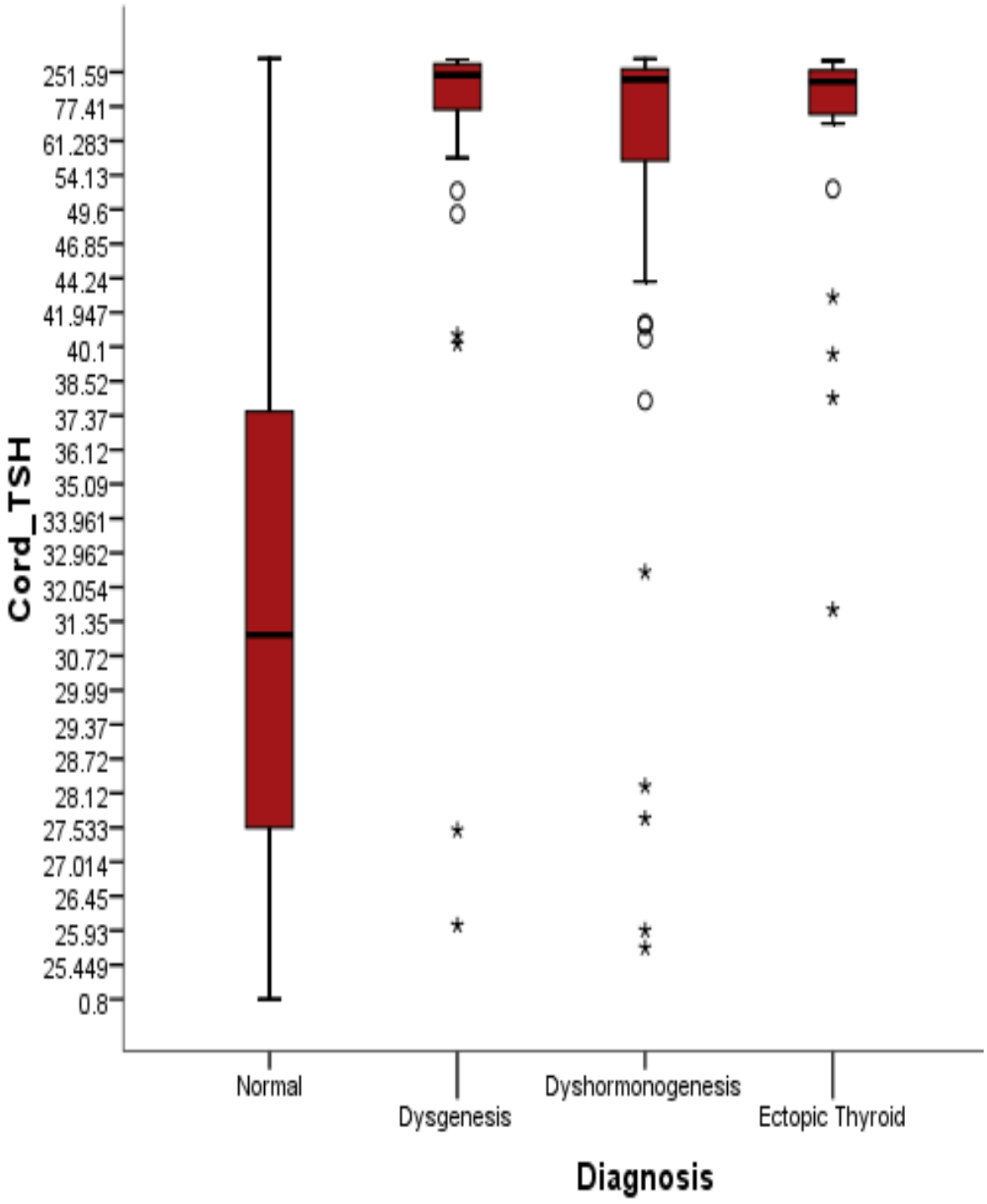


Figure 19. GG plot demonstrating cord TSH concentrations observed among various aetiologies of congenital hypothyroidism

FALSE NEGATIVE CASE

In the last 16 years, only 1 child who was screen negative(CBTSH11.8mIU/L)presented with clinical features congenital hypothyroidism and confirmed to have athyreosis.

False negative screen who later was confirmed with CH (n=1)	
GA wks/ B.wt kg	37+2/ 2.5
CB TSH mIU/L	11.8
Presentation with CH	Age 16 months:short stature myxedema, developmental delay
TSH mIU/L	>750
T4/FT4 (mcg/dl , ng/dl)	<0.3/ <0.1
Tc 99m scan	Thyroid agenesis

Total N screened	CBTSH (mIU/L)	Recalled	Re-sampled	Recall rate%	True positives	**PPV %	Sensitivity %	Specificity %
N= 14,742	20–25	293	195	1.3	0	0	0	98.8
N= 1,64,163	>25	5488	4224	2.57	123	2.9	99.2	97.5
	>26	4928	3824	2.32	119	3.1	99.2	97.8
	>27	4393	3434	2.09	117	3.4	99.1	98
	>28	3911	3070	1.87	117	3.8	99.1	98.2
	>29	3474	2728	1.66	117	4.2	99.1	98.4
	>30	3120	2465	1.5	117	4.7	99.1	98.5

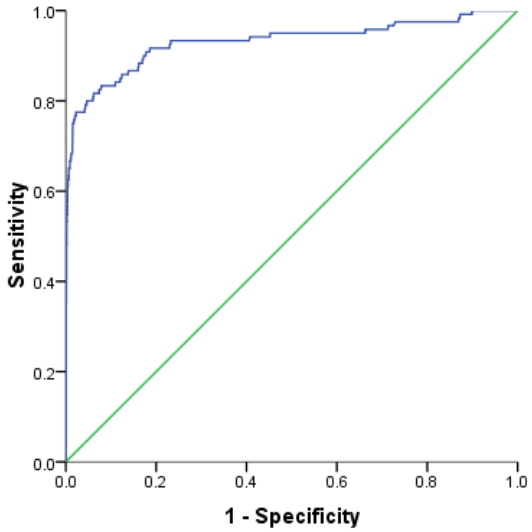
*Recall rate calculated based on actual number of babies resampled

**PPV: positive predictive value

Table 13.Sensitivity, specificity and PPV of babies with CBTSH between20-30mIU/L

Table 11 shows the sensitivity, specificity, positive predictive value and negative predictive value of CBTSH between 20-30mIU/L. CBTSH of 25mIU/L has a sensitivity of 99.2% and specificity of 97.5%. As the CBTSH increases to 30mIU/L, the sensitivity decreased to 99.1% and specificity improved to 98.5%. Recall rate with CBTSH of 25mIU/L was 2.57% and 1.5% with CBTSH of 30mIU/L.Only those who were re-sampled were included to calculate recall rate

Assumption: one false negative case



Area Under the Curve				
Area	**Std. Error[a]**	**Asymptotic Sig.[b]**	**Asymptotic 95% Confidence Interval**	
			Lower Bound	**Upper Bound**
.931	***.017***	***.000***	***.898***	***.963***

a.Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 20. ROC curve for CBTSH data >25mIU/L

The figure shown above depicts the ROC curve for CBTSH data above 25mIU/L. From the graph one can infer that the CBTSH has good sensitivity and is a suitable tool for screening for CH.

DISCUSSION

This study is primarily a retrospective analysis of the data from an ongoing newborn thyroid screening programme and is by far the largest cohort from a single institution in India.

The ideal sample for newborn thyroid screening is still controversial. While there are several advantages as well as disadvantages of using cord blood versus postnatal sample for screening, our experience has shown that using cord blood TSH, >99.25% of the hospital born babies were screened(Table 1). This includes 8-10% of the babies whose cord blood samples were missed and were recaptured prior to discharge from the hospital.CMC has to its advantage of being a single private hospital with all laboratory facilities in-house and TSH report available within 24 hours.This is a much higher coverage than reported by other centres in India using postnatal sampling at >24 hours of age (86.3% from Chandigarh , 73% from Lucknow)[63,64].This becomes particularly relevant as those who did not return for confirmatory sampling were ~ 23% in our institution(Table 1) comparable with similar data (15-30%) from other centres. In India, 70- 80 % mothers are discharged within 48 hours after delivery and the major reason for missed screening is early post-natal discharge. Post-discharge sampling relies on parents to bring back to hospital a “healthy normal” newborn baby for “blood” test. Unless we have a health care system with reliable & enthusiastic social/ health workers to visit the families at home, postnatal sampling using dried blood spot may not serve its purpose of being an effectiveuniversal newborn screening tool.

Another advantage with CB screening is the possibility for earlier recall, confirmation & treatment initiation compared to postnatal sampling .Excluding some babies with dyshormonogenesis who needed upto 3 blood tests for confirmation of CH, treatment was initiated at a mean age of 8.4 days after birth in our cohort as compared to 17.7 days in a screening programme using postnatal sampling[64]. In fact treatment was initiated by the end of 2nd week of life in > 90% of our cohort (data not shown). Our data was comparable to international data. In New Zealand, using postnatal DBS sample for screening, 23% of children were diagnosed within the first week of life and by the end of the 2nd and 3rd weeks, 80% and 90% children were diagnosed respectively[65]

The recall rates in screening programmes using CBTSH levels are higher because of the wide standard deviation in CBTSH levels. The corrected recall rate in our institution based on those who actually returned for confirmatory sampling was 2.54%. Of these 123 infants were confirmed to have primary CH. This means approximately 34 infants were recalled to confirm one infant with CH. In our institution, 60-70% of the patients are self-paying and subsidized investigation rates are available to the poor, therefore recall rate was not a major concern for us.Given the devastating consequences of missed or delayed initiation of treatment in congenital hypothyroidism, this “high” recall rate is still cost-effective. In a small cohort from Kerala using a CBTSH cut-off of 16.1mIU/L, the recall rate was 5.4%[67]

Our hospital based incidence is 1: 1346, which is comparable to the rest of India (1:1200 in Lucknow, 1: 1700 in Hyderabad , 1:1221 in Delhi) but higher than reported by most of the developed countries where it was 1:4000[21]

OVERALL CBTSH TREND

The mean CBTSH levels in our data between January 2004 and January 2017 was 9.186mIU/L. In a cohort of 450 babies, Prof Meena Desai reported a lower CBTSH level of 5·069±7·4 μ U/ml in full term babies and 7·88±3·77 μ U/ml in preterm babies from Mumbai in 1985[66].A recent data from Kerala also reported lower mean CBTSH level {7.82(5.42) μIU/mL}.

There was significant difference in the mean CBTSH concentration when compared to the previous year in the years 2006, 2009, 2011and 2012.The two main factors affecting CBTSH level are maternal iodine status and preterm births. Universal iodinisation of salt as part of the National iodine deficiency control program was launched in India in 1992.Although the vast majority of our population in Vellore uses iodised salt, we do not have any data from our institution on the iodine status of the mothers.

Another factor that affects the CBTSH level is prematurity. The mean CBTSH levels in preterm babies is 2 mIU/L(0.8-5.2) as compared to 9 mIU/L(1-17.4) in term babies[55]. Preterm babies also have a less marked TSH surge after birth[55].In fact, both false positive and false negative screen TSH levels are well documented in high risk neonates such as preterm, low birth weight and multiple births[68–70].

Another factor that could be attributed is the increased numbers of instrumental deliveries in our population which in itself is an independent risk factor for increased in the mean CBTSH as per our study(p=<0.001).This was also reported by another recent Indian study where the mode of delivery and perinatal stress factors were shown to have significant impact on CBTSH levels[71]..

CBTSH BETWEEN 20 – 25mIU/L

Analysis of babies born with CBTSH 20-24.99 showed an interesting fact. The mean age at which confirmatory sampling was done was earlier in the retrospective versus the prospective cohort(6.84±8.53 versus 10 ± 12.06 days). In addition while >96% of the recalled babies returned for resampling in 2001-2002, only 40 % returned in 2017. There are primarily two factors responsible for this drastic change. The retrospective cohort was during the initial few months of introduction of NBS in our institutionwhen CBTSH cut-off of 20 mIU/l was practiced as the **standard of care.** Therefore we were able to explain to the parents the importance of repeat sampling for confirmation of diagnosis. In the current scenario, with CBTSH of 25mIU/l being used as the standard cut-off , we had to recall parents as "part of a study" which was entirely voluntary and optional. In addition, in 2001, follow-up of abnormal TSH values was intense with even home visits by a social worker. Currently with no field staff/ social workers to pursue, the non-responder's families are contacted by telephone and or letter and the vast majority of families are unwilling to return for resampling a "healthy" baby. Focusing on improving awareness regarding the

usefulness of NBS for CH through mass media as well as home visits by health workers may improve the coverage.

This is an important observation in the context of advocating postnatal sampling as a screening test for CH in our population. There are many practical difficulties that families face in returning to the hospital such as distance from hospital, mode of conveyance in order to transport a newborn baby and in our study many patients did mention that once the baby is born they spend the next 3 months in their maternal home, which is usually in a different city as the hospital and returning for the blood test is difficult. In the developed countries, newborn screening is primarily carried out by health workers who collect dried blood spot at home, however this is difficult in our country where the population is large and the health system is overburdened as it is.Table 2 shows that majority of the babies who came back for repeat testing in the 20-25mIU/L group were term babies, of normal birth weight and were born by normal vaginal delivery. We may postulate that mothers who were post-operative(LSCS) may have had difficulty in travelling back to the hospital for resampling their babies.

195 babies with CBTSH 20-24.99 mIU/L were retested, none had congenital hypothyroidism. Overall babies with CBTSH 20-24.99mIU/L represented 2.6% of the births in 2001-2001 while it decreased to 1.6% of the total births in 2017.Thus lowering CBTSH cut-off for recall to any value between 20-< 25mIU/L would mean a recall of approximately additional 300 babies/ year which significantly increases the the cost of screening and cause unnecessary psychological stress in the parents . A study from UK reported a 126% increase in recall rate when screen TSH cut-off was

lowered to 6.1 from 10mU/L[70].Similar findings have been reported from India also: using a CBTSH cut-off of 16.1 , the recall rate was 5.4% in Kerala [72].

In the prospective cohort of babies with CBTSH 20-24.99 mIU/L , there was no correlation between CBTSH and factors such as birth weight, gestational age and mode of delivery. This could be attributed to the small sample size. .

CBTSH BETWEEN 25 – 30mIU/L

Over the last 17 year period between July 2001 to August 2017 CBTSH was performed on 1,65,637 babies of which 5,488 babies had a CBTSH concentration above 25mIU/L. The overall mean CBTSH was 39.71 ± 50.37mIU/L, with the CBTSH ranging from 25-1070mIU/L. When we divide this data into two groups ie.(i) Babies in whom CH was excluded and (ii) Babies in whom CH was diagnosed, as expected, the CBTSH in the first group is significantly lower than the second group(35.32±24.62 vs 240.04±218.78mIU/L).

There has been an increase in both the mean CBTSH levels as well as the number of babies diagnosed with CH between 2001-2004 and 2005-2008 (Table 8). This could be attributed to the increase in the number of deliveries each year and the proportionate increase in the babies who had a CBTSH above 25mIU/L. It is worth noting here that the number of deliveries was 7644 in the year 2001 which has now nearly doubled to 14,749 deliveries in the year 2016. Therefore with more babies

being screened each year, there is an increase in the number of babies diagnosed with CH.

Among the perinatal factors which affect CBTSH level, mode of delivery is one of the important factors. In our cohort, a significant correlation was seen between CBTSH and mode of delivery(correlation coefficient 0.062, p <0.001) while birth weight and gestational age did not show a significant correlation(Table 9). This is keeping with the finding of Amir-Mohammad et al in 2013 where they reported a significant correlation only between CBTSH and mode of delivery and not with gestational age, birth weight or APGAR at 5 mins[73].The increase the CBTSH particularly in instrumental deliveries may be explained by the increased perinatal stress that the newborn undergoes during a difficult delivery as compared to a normal delivery/elective caesarian section[74].

Among those who did not return for repeat testing is a small group of babies with CBTSH levels as high as 201mIU/L. These babies were severely depressed at birth and therefore the high CBTSH can be explained by the perinatal stress[74]. However we could not document a normal thyroid function in these babies as they either expired or were discharged against medical advice.

The commonest etiology of primary CH in most in the Caucasian population is thyroid dysgenesis[75]. However in our cohort the proportion of children with dyshormonogenesis, dysgenesis and ectopia were 44%, 30% and 21% respectively. The increased proportion of dyshormongenesis may be explained by prevalence ofconsanguinity in our population. Klett et al reported 24-36% dyshormonogenesis

35-42% ectopia and 22-42% dysgenesis in their cohort [76].Transient CH is known to occur with dyshormonogenesis. Interestingly despite having a greater proportion of children with dyshormonogenesis, permanence of CH was documented in > 80% of our cohort (data not shown).

The CBTSH level does not usually correlate with the severity of CH, for example, athyreosis which represents the most severe form of CH does not always have the highest CBTSH level[77]. In our cohort, the highest CBTSH level was observed in babies withthyroid dysgenesis followed by those with dyshormonogenesis and ectopia,with significant difference in each etiology((p=<0.001, Table 9).However one cannot predict the etiology of CH from the CBTSH level as the CBTSH levels varied greatly ineach etiology (dysgenesis 25.3-731mIU/L, dyshormonogenesis 27.5-750mIU/L, ectopia 31.6-750mIU/L). This was further evident from Figure 9 as CH was ruled out in 13 of the 93 babies with CBTSH >100mIU/L. This means that 14% of the babies in our cohort did not have CH even though they had a CBTSH as high as >100mIU/L. This gives further evidence to the fact that primary CH cannot be diagnosed on the basis of a single abnormal screen TSH value and it should be confirmed by repeat thyroid profile showing age appropriate elevated TSH and low Free T4 levels. .

THE IDEAL CBTSH FOR NEW BORN SCREENING

There is no real consensus on what is the ideal CBTSH for newborn screening of CH.This study has tried to address this issue by analysing the effect of lowering and increasing the current CBTSH cut-off of 25mIU/L in our screening program.In the 20-

25mIU/L group, there were no cases of CH and hence the sensitivity is zero(Table 12). Decreasing the CBTSH to 20mIU/L would mean that an additional 2%(approximately 300 babies) babies will have to be re-called for confirmatory sampling.Considering the fact that a total of 14,742 babies were screened, this number becomes relevant. Therefore, we postulate that lowering the CBTSH cut off<25mIU/L may not be cost-effective for our country.

In the last 16 years we had only one false negative case. This baby who had a CBTSH of 11.8 mIU/L returned at 16 months of age with florid congenital hypothyroidism. Thus false negativity may occur even in a robust screening programme and newborn screening cannot replace careful clinical examination. If clinical suspicion is high, biochemical tests have to be done irrespective of screen results.

Using a CBTSH cut off of 25mIU/L, the sensitivity was 99.2%, specificity was 97.5% and PPV was 2.9%. This level of sensitivity and specificity makes CBTSH of 25mIU/L , as is our current practice, an excellent choice as a screening test. If we increase the cut off from 25mIU/L to 30mIU/L, the specificity would increase and recall rate decrease by 1%, however 6 babies with CH who had CBTSH levels 25-30 mIU/L would have been missed(Figure 9). By the time these ‘missed” cases are diagnosed clinically, irreversible developmental delay may have occurred. The impact of a child with significant developmental delay on the family is enormous and cannot be quantified

One of the biggest problems with analyzing the sensitivity and specificity of the range of CBTSH levels (20-30 mIU/L) was verification bias well documented with such

screening tests[78]. The gold standard for diagnosing congenital hypothyoidism is by demonstrating elevated TSH level and low Free T4 level in the confirmatory sampling. However in our screening programme, only those with CBTSH > 25 mIU/L as per the existing practice and CBTSH > 20 during the study period had a confirmatory sample done and CH ruled out or confirmed. As only one child who was screen negative from our cohort presented with CH later on, in our analysis, we have included one as false negative on the assumption that all those who were screen negative and did not undergo the gold standard test were disease free. Therefore there is a verification bias in the analysis and this may have affected the actual sensitivity and specificity reported in this study.

Although we are aware of this limitation, it was not feasible for all children (screen negative and positive) to undergo the "gold standard" repeat sampling because of the practical difficulties and the huge expenditure involved. We have probably minimized the verification bias by resampling 195 children with CBTSH below our standard cut-off. The large sample size, longitudinal nature of our screening programme and a long follow-up of 16 years also may have minimized the verification bias.

There is no consensus on what is the ideal CBTSH for newborn screening for CH. The decision to increase or decrease the cut off should be made after consideration of many factors such as sensitivity, specificity, acceptable re-call rate for that population etc. While increasing the screen cut-off value reduces the recall rate it reduces the sensitivity of the test, in other words, may miss few children with CH. At the same time, should resource limited countries have a higher screen cut-off and hope to diagnose and treat the vast majority of children with CH while being aware that few

children with “mild CH” may have been missed?. With our experience over the last 16 years with a successful CBTSH based screening program we propose CBTSH of 25mIU/L as the ideal screen cut-off for our population.

India, being one of the fastest growing countries in terms of population and economy, a healthy population is most vital if it really needs to capitalize on this population dividend. As per the CES data of 2009, the overall number of institutional deliveries (rural + urban) have increased from 38.7%to 72.9%[56]. With this increase in the percentage of institutional deliveries, implementation of newborn screening would be another step in the right direction to ensure a healthy population.

Mental retardation(MR) as a condition not only affects the person having the disease but also causes significant psychological and financial stress to their family and care givers[79,80]. Congenital hypothyroidism, being one of the most preventable and easily treatable causes of MR, makes it a suitable candidate for universal new born screening.

CONCLUSIONS

1. More than 99% of the babies born in our institution were screened for congenital hypothyroidism using cord blood samples. With significant increase in the number of institutional deliveries in India, CBTSH is the most practical and cost effective screening tool for CH in our country..

2. Loweringthe CBTSH to 20mIU/L would mean that an additional 2%(approximately 300 babies) babies will have to be re-called for confirmatory sampling.

3. Therefore lowering the CBTSH cut off below 25mIU/L may not be cost-effective for our country.

4. Increasing the CBTSH cut-off to 30 mIU/L may lower the recall rate by 1%, however 6 babies with CH would have been missed. The devastating consequences of a late diagnosis of CH in these babies would have been enormous.

5. A CBTSH of 25mIU/L, with its high sensitivity and specificity, makes it an excellent choice as a newborn screening tool.

6. With the availability of simple, easy and low cost diagnostic and treatment options for congenital hypothyroidism, with an excellent outcome, universal newborn screening program should be initiated at the earliest in India. .

LIMITATIONS

1. In the initial years of our NBS, TSH levels were not performed in dilution, so we do not have absolute baseline values for few subjects.

2. Only 195 babies with CBTSH <25mIU/L had undergone confirmatory testing with the gold standard test. Therefore there is a verification bias in the analysis and this may have affected the actual sensitivity and specificity reported in this study. Resampling few screen negative babies, large sample size, longitudinal nature of our screening programme and a long follow-up of 16 years may have minimized the verification bias.

3. Missing data of some of the babies (gestational age, birth weight, mode of delivery) was obtained from registers and patient information available from computer scanned charts. In our institution, computerization of patient's records was done from 2010 onwards. Therefore some of the missing data prior to 2010could not be verified.

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ANNEXURES

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

March 03, 2017

Dr Praveen George Paul,
PG Registrar,
Department of Child Health -1,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population.
Dr Praveen George Paul Employment Number: 29357, Post graduate registrar, Paediatrics unit I, Dr Sarah Mathai, Employment Number: 14949, Paediatrics I, Ms Reka K Employment No.:32547, Biostatistics, Dr Joseph Bondu, Biochemistry.

Ref: IRB Min No: 10304 [OBSERVE] dated 12.10.2016

Dear Dr Praveen George Paul,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr Sarah Mathai, Dept. of Child Health -1, CMC, Vellore

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
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OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

March 03, 2017

Dr Praveen George Paul,
PG Registrar,
Department of Child Health -1,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population.
Dr Praveen George Paul Employment Number: 29357, Post graduate registrar, Paediatrics unit I, Dr Sarah Mathai, Employment Number: 14949, Paediatrics I, Ms Reka K Employment No.:32547, Biostatistics, Dr Joseph Bondu, Biochemistry.

Ref: IRB Min No: 10304 [OBSERVE] dated 12.10.2016

Dear Dr Praveen George Paul,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population" on October 12th 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Cv's of Drs. Praveen and Sarah Mathai.
3. Consent forms and Information Sheets.
4. No. of documents 1 - 3

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 12th 2016 in the BRTC Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
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Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal,Basic Medical Scientist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Santhanam Sridhar	MBBS, DCH, DNB	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MBBS, MD, DNB, PhD	Professor, Endocrinology, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10304 [OBSERVE] dated 12.10.2016 4 of 4

Proforma for newborns with cord TSH between 20mIU/L to 24.9mIU/L

1. Serial no :

2. Name :

3. Hospital number :

4. Sex : 1. Male 2. Female

5. Village / Town / City name :

6. Was maternal thyroid function test done during prenanacy: 1. Yes 2. No

7. Was mother on Thyroxine supplementation during pregnancy: 1. Yes 2. No

8. Use of iodised salt at home : 1. Yes 2. No

9. Family history of Thyroid disease: 1.Yes
   2. No

10. If yes, Specify _____

Patient information sheet

Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population

Why do we need this test?

Thyroid gland is a gland in the neck which produces thyroid hormones which are very important for the physical and mental development of children. Some children are born without a properly functioning thyroid gland and this condition is called congenital hypothyroidism (CH). If thyroid hormone supplements are started for these children within 2-3 weeks of birth their development is excellent. However it is difficult to diagnose congenital hypothyroidism at birth just by seeing the baby as the clinical features are very minimal at birth. Therefore it is important to screen all children at birth for CH before the clinical features appear. Very few hospitals in India screen newborn babies at birth for CH. All babies born in Christian Medical College Vellore are screened for CH since June 2001. The newborn screening is done by collecting few drops of blood from the baby's umbilical cord at birth (cord blood) and analyzing TSH level . Currently all babies with cord blood TSH > 25 Miu/l are called again for repeat tests and confirmation of diagnosis.

We are planning on a study to assess whether this cut-off level of 25 Miu/L is optimal. Therefore over the next one year we intend to recall all babies with cod blood TSH 20-25 Miu/L for repeat testing.

Do you think my baby may be affected?

When we ask you return on day three for repeat blood tests it does not mean that your baby definitely has the disease. It only means that screening test performed on the cord blood was higher than the accepted cut off and that your baby may have a chance of having congenital hypothyroidism. The diagnosis of congenital hypothyroidism can be made only after seeing the repeat blood tests.

What do we do for the test?

If your baby's cord blood TSH is between 20-25 you will be contacted for participation n this study. If you agree to paraticipate we will repeat a blood sample after 3 days of age. The blood sample will be collected by a paediatrician or an experienced nurse in our Project room located on the 5th floor of the ISSCC building. You may directly bring the baby to this location without any prior appointment.

Will we tell you the result of the test?

Yes. The results of the test will be told to you by telephone by the study team.

What happens if my baby has a positive test?

If the repeat testing is abnormal, your baby will be seen by a paediatric endocrinologist as soon as possible and treatment will be initiated.

Do I have to pay for the test?

No, as this test is being done as a part of a research project and it is done free of cost.

Could I opt out of the study at any point?

Yes, you could opt out at any point during the course of the study.

If you have any questions, then please contact me on:

Dr Praveen George Paul

PG Registrar

Department of Paediatrics unit I

CMC Vellore

Mob no : 9994882234

Informed Consent Form for Subjects

Ideal cord blood TSH cut-off level for mass newborn thyroid screening in the South Indian population

Number: _____

Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that ***the Sponsor of the clinical trial, others working on the Sponsor's behalf (delete as appropriate)***, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in

relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

TSH DATA			
DATE	UNIT	SERVICE NAME	RESULT
18-Oct-04	NB	TSH(NEONATAL)	3.86
18-Oct-04	NB	TSH(NEONATAL)	4.01
18-Oct-04	NB	TSH(NEONATAL)	3.18
18-Oct-04	NB	TSH(NEONATAL)	5.42
18-Oct-04	NB	TSH(NEONATAL)	11.2
18-Oct-04	NB	TSH(NEONATAL)	4.21
18-Oct-04	NB	TSH(NEONATAL)	
19-Oct-04	NB	TSH(NEONATAL)	6.95
19-Oct-04	NB	TSH(NEONATAL)	4.31
19-Oct-04	NB	TSH(NEONATAL)	9.74
19-Oct-04	NB	TSH(NEONATAL)	3.28
19-Oct-04	NB	TSH(NEONATAL)	4
19-Oct-04	NB	TSH(NEONATAL)	16.2
19-Oct-04	NB	TSH(NEONATAL)	
19-Oct-04	NB	TSH(NEONATAL)	16.9
19-Oct-04	NB	TSH(NEONATAL)	0.725
19-Oct-04	NB	TSH(NEONATAL)	23.4
19-Oct-04	NB	TSH(NEONATAL)	10.7
19-Oct-04	NB	TSH(NEONATAL)	5.51
19-Oct-04	NB	TSH(NEONATAL)	4.84
19-Oct-04	NB	TSH(NEONATAL)	5.74
20-Oct-04	NB	TSH(NEONATAL)	13.8
20-Oct-04	NB	TSH(NEONATAL)	5.91
20-Oct-04	NB	TSH(NEONATAL)	3.26
20-Oct-04	NB	TSH(NEONATAL)	3.56
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	9.39
20-Oct-04	NB	TSH(NEONATAL)	6.25
20-Oct-04	NB	TSH(NEONATAL)	8.43
20-Oct-04	NB	TSH(NEONATAL)	11.9
20-Oct-04	NB	TSH(NEONATAL)	9.46
20-Oct-04	NB	TSH(NEONATAL)	6.26
20-Oct-04	NB	TSH(NEONATAL)	6.69
20-Oct-04	NB	TSH(NEONATAL)	7.15
20-Oct-04	NB	TSH(NEONATAL)	10.9
20-Oct-04	NB	TSH(NEONATAL)	23.5
20-Oct-04	NB	TSH(NEONATAL)	2.63
20-Oct-04	NB	TSH(NEONATAL)	10.7
20-Oct-04	NB	TSH(NEONATAL)	12.9
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	
20-Oct-04	NB	TSH(NEONATAL)	3.85
20-Oct-04	NB	TSH(NEONATAL)	25
20-Oct-04	NB	TSH(NEONATAL)	3.91

20-Oct-04	NB	TSH(NEONATAL)	7.79
20-Oct-04	NB	TSH(NEONATAL)	12.4
21-Oct-04	NB	TSH(NEONATAL)	3.69
21-Oct-04	NB	TSH(NEONATAL)	2.99
21-Oct-04	NB	TSH(NEONATAL)	4.31
21-Oct-04	NB	TSH(NEONATAL)	3.02
21-Oct-04	NB	TSH(NEONATAL)	5.26
21-Oct-04	NB	TSH(NEONATAL)	4.15
21-Oct-04	NB	TSH(NEONATAL)	2.53
21-Oct-04	NB	TSH(NEONATAL)	3.82
21-Oct-04	NB	TSH(NEONATAL)	3.68
21-Oct-04	NB	TSH(NEONATAL)	5.23
21-Oct-04	NB	TSH(NEONATAL)	2.25
21-Oct-04	NB	TSH(NEONATAL)	9.1
21-Oct-04	NB	TSH(NEONATAL)	3.04
21-Oct-04	NB	TSH(NEONATAL)	5.11
21-Oct-04	NB	TSH(NEONATAL)	7.66
21-Oct-04	NB	TSH(NEONATAL)	6.91
21-Oct-04	NB	TSH(NEONATAL)	4.88
21-Oct-04	NB	TSH(NEONATAL)	36.7
22-Oct-04	NB	TSH(NEONATAL)	7.24
22-Oct-04	NB	TSH(NEONATAL)	5.51
22-Oct-04	NB	TSH(NEONATAL)	8.9
22-Oct-04	NB	TSH(NEONATAL)	8.69
22-Oct-04	NB	TSH(NEONATAL)	3.63
22-Oct-04	NB	TSH(NEONATAL)	4.33
22-Oct-04	NB	TSH(NEONATAL)	4.84
22-Oct-04	NB	TSH(NEONATAL)	
22-Oct-04	NB	TSH(NEONATAL)	
22-Oct-04	NB	TSH(NEONATAL)	10.3
22-Oct-04	NB	TSH(NEONATAL)	8.24
22-Oct-04	NB	TSH(NEONATAL)	6.17
22-Oct-04	NB	TSH(NEONATAL)	
22-Oct-04	NB	TSH(NEONATAL)	5.57
22-Oct-04	NB	TSH(NEONATAL)	4.92
22-Oct-04	NB	TSH(NEONATAL)	3.6
23-Oct-04	NB	TSH(NEONATAL)	6.56
23-Oct-04	NB	TSH(NEONATAL)	9.43
23-Oct-04	NB	TSH(NEONATAL)	10.9
23-Oct-04	NB	TSH(NEONATAL)	3.11
23-Oct-04	NB	TSH(NEONATAL)	3.31
24-Oct-04	NB	TSH(NEONATAL)	
25-Oct-04	NB	TSH(NEONATAL)	
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25-Oct-04	NB	TSH(NEONATAL)	6.53
25-Oct-04	NB	TSH(NEONATAL)	3.16
25-Oct-04	NB	TSH(NEONATAL)	4.14
25-Oct-04	NB	TSH(NEONATAL)	11.4
25-Oct-04	NB	TSH(NEONATAL)	10.7
25-Oct-04	NB	TSH(NEONATAL)	
25-Oct-04	NB	TSH(NEONATAL)	9.7
25-Oct-04	NB	TSH(NEONATAL)	10.2
25-Oct-04	NB	TSH(NEONATAL)	103
25-Oct-04	NB	TSH(NEONATAL)	4.22
25-Oct-04	NB	TSH(NEONATAL)	1.69
25-Oct-04	NB	TSH(NEONATAL)	34.4
25-Oct-04	NB	TSH(NEONATAL)	1.81
25-Oct-04	NB	TSH(NEONATAL)	12.5
25-Oct-04	NB	TSH(NEONATAL)	8.37
25-Oct-04	NB	TSH(NEONATAL)	6.02
25-Oct-04	NB	TSH(NEONATAL)	6.67
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26-Oct-04	NB	TSH(NEONATAL)	
26-Oct-04	NB	TSH(NEONATAL)	10.2
26-Oct-04	NB	TSH(NEONATAL)	12.7
26-Oct-04	NB	TSH(NEONATAL)	7.68
26-Oct-04	NB	TSH(NEONATAL)	
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26-Oct-04	NB	TSH(NEONATAL)	3.33
26-Oct-04	NB	TSH(NEONATAL)	5.69
26-Oct-04	NB	TSH(NEONATAL)	5.08
26-Oct-04	NB	TSH(NEONATAL)	2.54
26-Oct-04	NB	TSH(NEONATAL)	7.33
26-Oct-04	NB	TSH(NEONATAL)	6.11
27-Oct-04	NB	TSH(NEONATAL)	
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27-Oct-04	NB	TSH(NEONATAL)	
27-Oct-04	NB	TSH(NEONATAL)	
27-Oct-04	NB	TSH(NEONATAL)	7.64
27-Oct-04	NB	TSH(NEONATAL)	5.93
27-Oct-04	NB	TSH(NEONATAL)	8.9
27-Oct-04	NB	TSH(NEONATAL)	13.2
27-Oct-04	NB	TSH(NEONATAL)	4.41
27-Oct-04	NB	TSH(NEONATAL)	2.49
27-Oct-04	NB	TSH(NEONATAL)	5.64

27-Oct-04	NB	TSH(NEONATAL)	4.59
27-Oct-04	NB	TSH(NEONATAL)	5.13
27-Oct-04	NB	TSH(NEONATAL)	3.19
27-Oct-04	NB	TSH(NEONATAL)	3.93
27-Oct-04	NB	TSH(NEONATAL)	24
27-Oct-04	NB	TSH(NEONATAL)	9.93
27-Oct-04	NB	TSH(NEONATAL)	3.91
27-Oct-04	NB	TSH(NEONATAL)	4.06
27-Oct-04	NB	TSH(NEONATAL)	6
27-Oct-04	NB	TSH(NEONATAL)	4.77
27-Oct-04	NB	TSH(NEONATAL)	5.95
27-Oct-04	NB	TSH(NEONATAL)	4.15
28-Oct-04	NB	TSH(NEONATAL)	
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28-Oct-04	NB	TSH(NEONATAL)	
28-Oct-04	NB	TSH(NEONATAL)	
28-Oct-04	NB	TSH(NEONATAL)	6.79
28-Oct-04	NB	TSH(NEONATAL)	6.22
28-Oct-04	NB	TSH(NEONATAL)	8.42
28-Oct-04	NB	TSH(NEONATAL)	8.18
28-Oct-04	NB	TSH(NEONATAL)	7.94
28-Oct-04	NB	TSH(NEONATAL)	4.4
28-Oct-04	NB	TSH(NEONATAL)	4.38
28-Oct-04	NB	TSH(NEONATAL)	4.34
28-Oct-04	NB	TSH(NEONATAL)	3.35
28-Oct-04	NB	TSH(NEONATAL)	
28-Oct-04	NB	TSH(NEONATAL)	4.81
28-Oct-04	NB	TSH(NEONATAL)	1.43
28-Oct-04	NB	TSH(NEONATAL)	4.71
28-Oct-04	NB	TSH(NEONATAL)	3.15
28-Oct-04	NB	TSH(NEONATAL)	7.53
28-Oct-04	NB	TSH(NEONATAL)	38
28-Oct-04	NB	TSH(NEONATAL)	3.72
28-Oct-04	NB	TSH(NEONATAL)	5.98
29-Oct-04	NB	TSH(NEONATAL)	12.4
29-Oct-04	NB	TSH(NEONATAL)	5.12
29-Oct-04	NB	TSH(NEONATAL)	5.91
29-Oct-04	NB	TSH(NEONATAL)	7.9
29-Oct-04	NB	TSH(NEONATAL)	6.43
29-Oct-04	NB	TSH(NEONATAL)	3.91
29-Oct-04	NB	TSH(NEONATAL)	12.1
29-Oct-04	NB	TSH(NEONATAL)	8.51
29-Oct-04	NB	TSH(NEONATAL)	6.42
29-Oct-04	NB	TSH(NEONATAL)	26.1
29-Oct-04	NB	TSH(NEONATAL)	3.24
29-Oct-04	NB	TSH(NEONATAL)	9.22
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29-Oct-04	NB	TSH(NEONATAL)	4.28
29-Oct-04	NB	TSH(NEONATAL)	3.9
29-Oct-04	NB	TSH(NEONATAL)	7.56
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29-Oct-04	NB	TSH(NEONATAL)	3.91
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29-Oct-04	NB	TSH(NEONATAL)	11.9
29-Oct-04	NB	TSH(NEONATAL)	8.13
29-Oct-04	NB	TSH(NEONATAL)	4.2
29-Oct-04	NB	TSH(NEONATAL)	15.6
29-Oct-04	NB	TSH(NEONATAL)	10.2
30-Oct-04	NB	TSH(NEONATAL)	
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30-Oct-04	NB	TSH(NEONATAL)	
30-Oct-04	NB	TSH(NEONATAL)	
30-Oct-04	NB	TSH(NEONATAL)	4.12
30-Oct-04	NB	TSH(NEONATAL)	4.47
30-Oct-04	NB	TSH(NEONATAL)	7.42
30-Oct-04	NB	TSH(NEONATAL)	14.1
30-Oct-04	NB	TSH(NEONATAL)	7.84
30-Oct-04	NB	TSH(NEONATAL)	5.94
30-Oct-04	NB	TSH(NEONATAL)	3.59
30-Oct-04	NB	TSH(NEONATAL)	3.38
30-Oct-04	NB	TSH(NEONATAL)	9.86
30-Oct-04	NB	TSH(NEONATAL)	8.8
30-Oct-04	NB	TSH(NEONATAL)	5.61
30-Oct-04	NB	TSH(NEONATAL)	9.73
31-Oct-04	NB	TSH(NEONATAL)	
31-Oct-04	NB	TSH(NEONATAL)	
31-Oct-04	NB	TSH(NEONATAL)	
31-Oct-04	NB	TSH(NEONATAL)	
31-Oct-04	NB	TSH(NEONATAL)	7.8
31-Oct-04	NB	TSH(NEONATAL)	4.22
31-Oct-04	NB	TSH(NEONATAL)	10.4
31-Oct-04	NB	TSH(NEONATAL)	3.66
31-Oct-04	NB	TSH(NEONATAL)	7.71
31-Oct-04	NB	TSH(NEONATAL)	25.4
31-Oct-04	NB	TSH(NEONATAL)	8.4
1-Nov-04	NB	TSH(NEONATAL)	
1-Nov-04	NB	TSH(NEONATAL)	
1-Nov-04	NB	TSH(NEONATAL)	
1-Nov-04	NB	TSH(NEONATAL)	
1-Nov-04	NB	TSH(NEONATAL)	10.1
1-Nov-04	NB	TSH(NEONATAL)	6.17
1-Nov-04	NB	TSH(NEONATAL)	2.24
1-Nov-04	NB	TSH(NEONATAL)	6.57

1-Nov-04	NB	TSH(NEONATAL)	6.32
1-Nov-04	NB	TSH(NEONATAL)	3.75
1-Nov-04	NB	TSH(NEONATAL)	21.5
1-Nov-04	NB	TSH(NEONATAL)	10.6
1-Nov-04	NB	TSH(NEONATAL)	6.04
1-Nov-04	NB	TSH(NEONATAL)	7.51
1-Nov-04	NB	TSH(NEONATAL)	5.92
1-Nov-04	NB	TSH(NEONATAL)	3.86
1-Nov-04	NB	TSH(NEONATAL)	4.44
1-Nov-04	NB	TSH(NEONATAL)	13.5
1-Nov-04	NB	TSH(NEONATAL)	1.89
1-Nov-04	NB	TSH(NEONATAL)	6.57
1-Nov-04	NB	TSH(NEONATAL)	3.6
1-Nov-04	NB	TSH(NEONATAL)	16.4
1-Nov-04	NB	TSH(NEONATAL)	1.89
1-Nov-04	NB	TSH(NEONATAL)	2.61
1-Nov-04	NB	TSH(NEONATAL)	11.6
1-Nov-04	NB	TSH(NEONATAL)	12.9
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	11.5
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	8.8
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	
2-Nov-04	NB	TSH(NEONATAL)	24.5
2-Nov-04	NB	TSH(NEONATAL)	11.3
2-Nov-04	NB	TSH(NEONATAL)	14.3
2-Nov-04	NB	TSH(NEONATAL)	6.52
2-Nov-04	NB	TSH(NEONATAL)	7.06
2-Nov-04	NB	TSH(NEONATAL)	5.7
2-Nov-04	NB	TSH(NEONATAL)	4.43
2-Nov-04	NB	TSH(NEONATAL)	10.9
2-Nov-04	NB	TSH(NEONATAL)	6.46
2-Nov-04	NB	TSH(NEONATAL)	7.68
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	

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3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	10.8
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
3-Nov-04	NB	TSH(NEONATAL)	
4-Nov-04	NB	TSH(NEONATAL)	4.79
4-Nov-04	NB	TSH(NEONATAL)	29.5
4-Nov-04	NB	TSH(NEONATAL)	12
4-Nov-04	NB	TSH(NEONATAL)	16.1
4-Nov-04	NB	TSH(NEONATAL)	3.24
4-Nov-04	NB	TSH(NEONATAL)	3.36
4-Nov-04	NB	TSH(NEONATAL)	4.48
4-Nov-04	NB	TSH(NEONATAL)	8.59
4-Nov-04	NB	TSH(NEONATAL)	16.8
4-Nov-04	NB	TSH(NEONATAL)	8.26
4-Nov-04	NB	TSH(NEONATAL)	15.1
4-Nov-04	NB	TSH(NEONATAL)	5.37
4-Nov-04	NB	TSH(NEONATAL)	4.36
4-Nov-04	NB	TSH(NEONATAL)	5.63
4-Nov-04	NB	TSH(NEONATAL)	42.4
4-Nov-04	NB	TSH(NEONATAL)	2.89
4-Nov-04	NB	TSH(NEONATAL)	5.54
4-Nov-04	NB	TSH(NEONATAL)	6.44
4-Nov-04	NB	TSH(NEONATAL)	2.75
4-Nov-04	NB	TSH(NEONATAL)	5.47
4-Nov-04	NB	TSH(NEONATAL)	7.15
4-Nov-04	NB	TSH(NEONATAL)	2.29
4-Nov-04	NB	TSH(NEONATAL)	10
4-Nov-04	NB	TSH(NEONATAL)	14.2
4-Nov-04	NB	TSH(NEONATAL)	4.03
4-Nov-04	NB	TSH(NEONATAL)	6
4-Nov-04	NB	TSH(NEONATAL)	6.68
4-Nov-04	NB	TSH(NEONATAL)	12.8

4-Nov-04	NB	TSH(NEONATAL)	4.58
4-Nov-04	NB	TSH(NEONATAL)	5.12
5-Nov-04	NB	TSH(NEONATAL)	
5-Nov-04	NB	TSH(NEONATAL)	5.79
5-Nov-04	NB	TSH(NEONATAL)	6.46
5-Nov-04	NB	TSH(NEONATAL)	4.02
5-Nov-04	NB	TSH(NEONATAL)	3.59
5-Nov-04	NB	TSH(NEONATAL)	4.36
5-Nov-04	NB	TSH(NEONATAL)	3.48
5-Nov-04	NB	TSH(NEONATAL)	5.78
5-Nov-04	NB	TSH(NEONATAL)	2.59
5-Nov-04	NB	TSH(NEONATAL)	9.31
5-Nov-04	NB	TSH(NEONATAL)	5.08
5-Nov-04	NB	TSH(NEONATAL)	655
5-Nov-04	NB	TSH(NEONATAL)	14.5
5-Nov-04	NB	TSH(NEONATAL)	6.1
5-Nov-04	NB	TSH(NEONATAL)	20.8
6-Nov-04	NB	TSH(NEONATAL)	
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6-Nov-04	NB	TSH(NEONATAL)	
6-Nov-04	NB	TSH(NEONATAL)	
6-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
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7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
7-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	7.14
8-Nov-04	NB	TSH(NEONATAL)	5.48
8-Nov-04	NB	TSH(NEONATAL)	4.22
8-Nov-04	NB	TSH(NEONATAL)	11.7
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	
8-Nov-04	NB	TSH(NEONATAL)	3.96
8-Nov-04	NB	TSH(NEONATAL)	4.56
8-Nov-04	NB	TSH(NEONATAL)	5.69
8-Nov-04	NB	TSH(NEONATAL)	2.11
8-Nov-04	NB	TSH(NEONATAL)	8.86
8-Nov-04	NB	TSH(NEONATAL)	23
9-Nov-04	NB	TSH(NEONATAL)	6.3
9-Nov-04	NB	TSH(NEONATAL)	5.9
9-Nov-04	NB	TSH(NEONATAL)	4.4
9-Nov-04	NB	TSH(NEONATAL)	5.7
9-Nov-04	NB	TSH(NEONATAL)	8.2
9-Nov-04	NB	TSH(NEONATAL)	5.6
9-Nov-04	NB	TSH(NEONATAL)	
9-Nov-04	NB	TSH(NEONATAL)	
9-Nov-04	NB	TSH(NEONATAL)	6.41

1	979558F	11/1/2015	9.17	1	36	2620	2	29.01
1	758563F	4/8/2014	2.43	1	38.2	2800	2	29.014
1	490153F	6/6/2013	4.07	1	38.2	2920	2	29.027
1	934392F	12/18/2014	4.49	1	39	3180	3	29.027
2	487567D	7/9/2009	2.5	2	39.2	3120	4	29.03
2	753714D	8/11/2010	3.18	2				29.03
2	788881D	10/5/2010	6.53	2	35	1080	2	29.03
1	028786F	9/15/2011	5.02	2	40.6	2880	4	29.03
2	568192G	7/20/2017	10.24	1	37.6	2630	3	29.03
1	352341D	15-Nov-08			38	2700	2	29.04
1	352365D	16-Nov-08			40.2	3460	2	29.05
2	730491D	7/15/2010	3.13	1		3580		29.05
1	992451D	8/4/2011	10.1	1	40.4	3070	4	29.05
1	999531C	10-Apr-07			47.4	3330	3	29.06
1	180785F	4/17/2012	10.29	2	40	3620	3	29.06
1	960555F	6/26/2015	12.49	1	38.1	2690	4	29.063
1	695796F	11/17/2013	2.02	2	39.5	2140	2	29.068
2	911926F	7/14/2014	2.31	2	38.6	2800	2	29.073
2	518456D	8/21/2009	12.59	2	40.5	3540	2	29.08
2	415760F	3/2/2013	4.16	2	39	3350	2	29.081
1	401964F	2/20/2013	3.03	1	37.2	2940	2	29.087
1	788522D	9/27/2010	1.19	2		3280		29.09
2	513460G	7/9/2016	6	2	37.6	2830	2	29.09
1	203211C	7-Sep-02			33.4	3040	2	29.1
1	260298C	5-Feb-03			38	3100	2	29.1
1	298773C	26-May-03			39.6	2620	2	29.1
1	379210C	1-Nov-03			39.2	2300	2	29.1
1	695563C	10-Oct-05			40.1	3390	2	29.1
1	722369C	9-Nov-05			40.1	3500	2	29.1
2	121451D	22-Oct-07			39.2	3080	2	29.1
1	248464D	6-Jun-08			38.6	3320	2	29.1
1	314356D	18-Sep-08			39.2	3020	2	29.1
1	901841D	3/21/2011	9.57	1	37.2	2980	2	29.1

1	901992D	3/25/2011	5.34	1	38.6	3240	2	29.1
1	028716F	9/14/2011	9.51	1	40.1	3280	2	29.1
1	174069F	4/7/2012	10.48	1	35.4	2440	2	29.1
1	507300G	5/22/2016	12.4	1	37.6	2300	2	29.1
1	938757F	2/20/2015	5.2	2	37.5	2760	4	29.105
2	618145F	7/16/2013	6.35	2	39.6	3920	2	29.108
1	591768D	12/3/2009	12.24	1	38.6	2980	3	29.11
1	591846D	12/4/2009	6.15	2	40.2	3720	2	29.11
2	969324F	8/29/2015	6.28	1	39.2	284	2	29.11
2	908815F	6/19/2014	11.36	1	36.4	2020	1	29.111
2	127262D	29-Oct-07			39.6	2540	3	29.12
1	835802D	12/1/2010	2.32	2	39.3	3370	2	29.12
1	853728D	12/31/2010	8.49	1		3100		29.12
1	059331F	10/26/2011	7.47	1	37.2	2920	3	29.13
1	156392F	3/16/2012	2.55	1	36	2420	2	29.14
2	918630F	9/13/2014	12.42	2	38.3	2730	2	29.14
2	490125F	6/5/2013	11.07	1	39	3010	2	29.141
1	108868D	5-Oct-07			40.8	3000	2	29.15
2	956938F	6/16/2015	6.42	2	39.5	3340	4	29.152
1	992179D	7/28/2011	7.47	2	38.2	2580	1	29.16
1	938687F	2/17/2015	10.26	1	37.1	2520	2	29.167
2	875765D	2/13/2011	4.23	1	38.3	4060		29.17
1	553269G	4/4/2017	11.04	1	37.2	2860	4	29.17
2	918880F	9/17/2014	3.17	1	38.5	2800	3	29.175
2	338082F	11/5/2012	1.05	2	38.3	3030	4	29.178
1	908097D	3/27/2011	11.2	2	40.3	3060	2	29.18
2	618282F	7/19/2013	1.51	2	37	2680	2	29.182
2	406104D	18-Feb-09			41	3100	2	29.19
2	953130F	5/12/2015	7.15	1	39.4	3660	4	29.193
2	073862C	31-Oct-01			40.4	2660	2	29.2
2	073949C	3-Nov-01			37.2	2680	2	29.2
1	170237C	21-Jun-02			40.6	3620	3	29.2
2	379318C	4-Nov-03			35.3	2040	2	29.2
1	449173C	9-Apr-04			38.4	2940	2	29.2
1	624918C	26-May-05			37.1	2600	2	29.2
1	674991D	4/28/2010	10.46	1		2800		29.2
2	724698D	7/5/2010	9.22	1	39	2780	2	29.2
1	946059D	5/22/2011	3.16	1	39.4	3200	4	29.2
1	107939F	1/7/2012	7.29	1	39.4	2870		29.2
1	911932F	7/14/2014	5.16	2	38.6	2920	2	29.201
2	695841F	11/18/2013	5.2	2	40.3	3030	2	29.206
1	522576G	9/15/2016	5.02	2	38.2	2490	2	29.21

2	308366D	7-Sep-08			36.3	2160	2	29.22
1	002939F	8/15/2011	9.57	2	40.2	3380	1	29.22
1	059279F	10/25/2011	3.1	2	40.2	3740	3	29.22
1	989663F	1/5/2016	3.17	2	38.5	2725	2	29.22
2	555199G	4/26/2017	2.23	1	39.2	3470	2	29.22
1	911503F	7/3/2014	11.47	2	39.5	3280	4	29.226
2	938628F	2/14/2015	5.2	2	38.5	2630	2	29.227
2	137523D	17-Nov-07			35.1	2160	2	29.23
2	374829F	1/2/2013	11.45	2	38.4	2810	4	29.231
2	118117D	15-Oct-07			39.4	2520	2	29.24
1	397806D	28-Jan-09			36	2640	2	29.24
1	770571D	9/4/2010	12.29	2		2020		29.24
1	741007F	1/3/2014	8.02	1	40.1	3240	2	29.24
2	657276F	9/10/2013	4.52	1	39.2	2560	3	29.245
2	200251F	5/17/2012	4.08	2	38.2	2670	2	29.252
2	389924F	2/2/2013	8.27	1	39.6	2800	2	29.256
2	927268F	11/4/2014	6.33	2	37.5	2680	4	29.267
2	930033F	11/19/2014	10.15	1	39.2	2880	2	29.274
2	522565G	9/15/2016	1.31	2	38.4	2810	2	29.28
1	927049F	10/30/2014	8.36	2	40	2590	2	29.283
1	821979D	11/16/2010	8.33	1		3200		29.29
1	553652G	4/14/2017	10.01	1	38.6	2900	2	29.29
1	231245C	15-Nov-02			39.5	2630	1	29.3
1	260588C	21-Feb-03			38.2	1700	2	29.3
1	624057C	21-Apr-05			40.4	3160	1	29.3
1	695659C	13-Oct-05			40.1	1800	1	29.3
1	739075C	6-Dec-05			36.1	3600	2	29.3
1	929060C	19-Nov-06			40.6	2760	2	29.3
1	290339D	12-Aug-08			40.1	3280	2	29.3
1	318253D	22-Sep-08			40.5	3120	3	29.3
2	361345D	26-Nov-08			35.5	1800	1	29.3
2	378856D	28-Dec-08			37.2	2600	2	29.3
2	429470D	29-Mar-09			40.5	3000	2	29.3
1	632493D	2/13/2010	8.51	2	39	3040	2	29.3

2	753975D	8/18/2010	10.59	1		3240		29.3
1	082152F	11/28/2011	3.48	2	39.1	2350	2	29.3
1	758099F	3/29/2014	12.22	1	37	2380	2	29.3
1	107101G	3/8/2016	11.13	2	37.5	2470	4	29.3
1	956268F	6/4/2015	1.53	1	38.3	2540	2	29.304
2	969972F	9/10/2015	5.04	1	40.4	2515	2	29.31
1	971063F	9/12/2015	8.2	1	37.5	2850	4	29.31
2	522558G	9/15/2016	11.29	1	39	2480	2	29.31
1	441724F	3/30/2013	1.5	2	39.4	3160	2	29.319
2	194762F	5/5/2012	3.54	2	38.3	2670	4	29.32
2	519692G	8/28/2016	9.26	2	40	2960	4	29.32
1	194926F	5/9/2012	3.45	2	39.1	2680	4	29.329
1	996662F	2/25/2016	12.46	2	37.3	2800	2	29.33
2	535482G	12/2/2016	12.41	2	38.2	2360	4	29.33
1	451149D	3-May-09			39.1	2920	2	29.35
2	943183F	3/27/2015	9.24	1	38.3	2740	1	29.363
2	552001G	3/3/2017	11.15	1	38.4	3520	2	29.37
2	028778F	9/15/2011	12.41	2	39.5	3120	2	29.38
2	207270F	5/25/2012	12.58	2	40.1	3250	3	29.389
1	918293F	9/5/2014	6.41	1	37.6	3380	4	29.394
1	741019F	1/3/2014	12.55	2	40.1	3300	4	29.396
1	060946C	23-Sep-01			37	3520	2	29.4
1	090802C	6-Dec-01			40.5	2640	2	29.4
2	170610C	8-Jul-02			40.4	3060	2	29.4
2	309997C	11-Jul-03			40	0	2	29.4
1	379455C	8-Nov-03			41.1	2580	2	29.4
2	605190C	15-Mar-05			38.4	2790	1	29.4
1	624216C	29-Apr-05			37.4	2820	2	29.4
2	681068C	18-Aug-05			38	2600	2	29.4
2	695109C	23-Sep-05			37.1	0	2	29.4
1	920329C	9-Nov-06			39	2870	2	29.4
1	940196C	10-Dec-06			37.1	1660	1	29.4
1	957556C	10-Jan-07			39.5	2560	2	29.4
2	196397D	7-Mar-08			38	2640	2	29.4

1	270811D	8-Jul-08			39.3	3240	2	29.4
1	290451D	15-Aug-08			37.4	3420	2	29.4
1	335964D	21-Oct-08			39	3100	2	29.4
2	526484D	8/30/2009	6.5	2	39	2860	2	29.4
1	993876F	2/5/2016	1.51	2	38.3	2720	3	29.4
2	109483G	4/10/2016	6.57	1	36.5	2360	2	29.4
1	529430G	10/21/2016	6.38	2	38.2	2850	2	29.4
1	535163G	11/25/2016	1.57	1	40.3	2900	4	29.4
2	810978D	10/30/2010	5.01	2		2800		29.42
1	308507D	11-Sep-08			40	3380	2	29.43
1	996126F	2/12/2016	4.45	2	39	3100	1	29.43
1	906567F	5/24/2014	6.43	1	39.5	2930	2	29.44
1	921595F	10/1/2014	11.16	1	35.1	1920	2	29.45
2	509420G	6/16/2016	8.43	1	37.5	2685	2	29.45
2	930281F	11/24/2014	4.15	1	38.3	2930	4	29.455
1	985038F	11/29/2015	10.2	1	40.3	2930	4	29.48
2	200372F	5/19/2012	3.07	2	40.6	3120	1	29.488
2	119847F							29.49
1	695805F	11/17/2013	10.29	2	40.4	3220	2	29.494
1	406990F	2/28/2013	11.19	1	40	3230	2	29.497
2	060726C	15-Sep-01			40	2460	2	29.5
1	090621C	28-Nov-01			40.1	2660	2	29.5
2	247563C	3-Jan-03			40.6	3960	2	29.5
1	309919C	9-Jul-03			40.5	3050	2	29.5
2	408927C	14-Feb-04			33.2	3900	1	29.5
1	783668C	2-Apr-06			39.6	3460	2	29.5
1	839608C	27-Jun-06			38.5	2390	2	29.5
2	840953C	15-Jul-06			41.1	3200	2	29.5
1	879359C	27-Aug-06			34	1980	2	29.5
1	929419C	30-Nov-06			36.3	2600	1	29.5
1	982569C	4-Mar-07			39.6	3570	2	29.5
1	982599C	5-Mar-07			39.4	3150	2	29.5

2	993615C	24-Mar-07			40.3	2870	2	29.5
1	993882C	4-Apr-07			40.2	2510	2	29.5
1	108817D	2-Oct-07			39.2	2680	2	29.5
2	241392D	22-May-08			38.2	2620	2	29.5
1	355434D	20-Nov-08			37.6	2800	2	29.5
1	661831D	3/30/2010	11.52	2		3880		29.5
1	927318F	11/5/2014	2.24	2	39.1	2480	2	29.506
1	918160F	9/2/2014	1.38	2	38.3	3400	1	29.509
2	927002F	10/29/2014	2.41	2	39.2	2820	2	29.514
1	338184F	11/8/2012	4.42	1	37.3	2950	3	29.515
1	934735F	12/25/2014	12.41	1	37.4	3030	2	29.516
1	830911D	11/26/2010	4.19	2		2880		29.52
1	509825G	6/26/2016	5.24	1	36.5	2420	4	29.52
2	553228G	4/3/2017	11.44	1	38.4	2682	2	29.52
2	560058G	5/15/2017	8.09	1	40.1	3120	2	29.52
1	750489D	8/5/2010	12.33	1		3440		29.53
2	126794F	2/3/2012	1.16	2	38.1	2200		29.53
1	458338F	4/28/2013	4.32	2	39.4	3390	2	29.53
2	514533D	8/3/2009	2.47	2	39	3800	2	29.54
1	144681D	2-Dec-07			40.3	3100	2	29.55
2	497184F	6/17/2013	5.33	2	39.4	2580	4	29.551
1	299363D	24-Aug-08			40.1	3280	2	29.56
1	591925D	12/6/2009	5.19	2	38.5	3020	1	29.56
2	770578D	9/4/2010	8.52	2		2700		29.56
1	064922F	11/3/2011	4.14	2	40.1	3960	3	29.56

11/4/2015	2.369	18.1	1.74	3
6/10/2013	5.08	18.8	2.03	4
12/22/2014	2.933	10.9	1.51	4
7/18/2009	4.18	17.2	2.02	9
9/19/2011	0.6	18.6	1.8	4
7/24/2017	4.191	14.4	1.91	4
	5.8	13.2	1.72	
	1.81	14.2	1.75	
8/8/2011	3.28	4.3	0.76	4
4/20/2012	10.67	20.5	2.83	3
7/6/2015	1.972	15.9	1.56	10
7/19/2014	0.32	12.8	1.37	5
3/19/2013	2.915	12.8	1.54	17
3/11/2013	4.606	8.4	1.57	19
9/30/2010	10.9	19.6	2.57	3
	9.36	8.8	1.53	
	1.54	15.6	1.95	
	2.8	13.1	1.55	
	29.4	5.81	1	
	15.1	15	2.21	
	4.39	13.7	2.39	
	0.64			
	5.1	9.4	1.53	
3/30/2011	1.44	14.9	1.61	9

4/10/2012	1.26	11.2	1.16	3
2/23/2015	1.115	17	2.51	3
7/19/2013		21.6	2.12	3
12/15/2009	4.11	16.9	1.21	12
12/15/2009	4.11	16.9	1.21	11
9/10/2015	700.3	18	1.64	12
6/27/2014	4.938	11.6	1.29	8
	0.32	8.2	1.56	
12/10/2010	2.1	18.8	18.2	9
1/6/2011	5.99	18.2	1.61	6
11/5/2011	5.01	18	1.73	10
3/19/2012	0.74	15.8	2.17	3
9/16/2014	0.949	16.7	1.78	3
6/8/2013	5.194	14.6	2.2	3
	18.9	11.2	1.3	
8/1/2011	0.35	10.8	1.49	4
2/20/2015	1.5	20.1	1.9	3
2/16/2011	2.14	21.9	2.31	3
4/18/2017	4.038	13.9	1.6	14
9/20/2014	4.126			3
11/9/2012	0.204	11.8	1.39	4
3/31/2011	1.92	19.7	1.85	4
7/24/2013	1.713	0.3	0.48	5
5/15/2015	2.623			3
	1	15.2	2.16	
	2.7	12.3	1.6	
	2.69	11.7	1.58	
	4.45	7.87	8.93	
	3.9	8.6	1.2	
5/1/2010	2.25	13.9	1.88	3
7/9/2010	1.52	10.2	1.38	4
5/25/2011	1	13.4	1.58	3
1/14/2012	2.93	15.8	1.81	7
7/22/2014	2.623	18.1	2	8
9/21/2016	1.123	8.6	1.52	6

	0.85	7.9	1.2	
8/19/2011	9.3	20.8	2.4	4
10/28/2011	12.33			3
1/9/2016	0.561	11.7	1.55	4
4/29/2017	1.618	13.2	1.32	3
7/7/2014	3.82	14.7	1.46	4
2/18/2015	0.537	24.4	2.35	4
	1.36	9.4	1.44	
1/6/2013	1.854	25.3	1.91	4
	8.27	9.4	1.2	
9/8/2010	0.5	10.2	1.52	4
1/6/2014				3
9/16/2013	4.752	16.3	1.72	6
5/21/2012	1.905	8.3	1.11	4
2/8/2013	6.276	14.5	1.39	6
11/7/2014	1.58	19.3	1.83	3
9/19/2016	2.352	15.2	1.59	4
11/3/2014	1.176	15.3	1.96	4
11/20/2010	1.49	13.7	1.45	4
	7.1	16.7	1.6	
	2.94	10.2	1.79	
	6	6.69	1.3	
	4.43	15.5	2.07	
	5.5	8.1	1.3	
2/17/2010	1.28	12.3	1.82	4

8/31/2010	6.06	16.2	1.89	13
12/2/2011	4.9	13.8	2	4
9/19/2015	4.238	10.9	1.6	7
9/23/2016	3.78	13.4	1.64	8
5/8/2012	1.928	20.7	1.99	3
8/31/2016	5.828	13.7	1.4	3
5/21/2012	1.024	11.4	1.23	12
12/5/2016	4.141	20.3	2.62	3
	10.13	16.3	2.13	
3/7/2017	3.364	15.8	1.79	4
9/20/2011	7.6	11.9	1.7	5
5/29/2012	2.67	13.4	1.37	4
9/15/2014	2.745	13.9	1.09	10
1/6/2014	1.825	19.6	1.95	3
	1.13	11.8	1.15	
	1.5	16	2.2	
	3.98	11.8	1.79	
	7.49	8.55	1.71	
	19.4	6.5	0.93	
	1.32	17.5	2.17	
	0.46	10.9	1.4	

	9.2	19.6	1.81	
	1.61	11.9	1.59	
	12.22	9.7	1.48	
9/7/2009	1.18	15.2	1.43	8
2/8/2016	1.836	17.4	2.58	3
4/21/2016	6.55	15.2	1.6	11
10/25/2016	0.863	15.1	1.78	4
11/28/2016	2.383			3
	3.65	11.7	2.07	
2/15/2016	3.479	17.4	2.04	3
10/4/2014	3.31	13.3	2.01	3
6/19/2016			1.34	3
11/28/2014	2.634	15.2	1.65	4
12/2/2015	1.018	14	1.85	3
5/24/2012	1.844	12.3	1.3	5
1/31/2012	1.04	11.5	1.54	
	5	14.3	2.43	
	4.34	13.5	1.65	
	4.36	1.95	0.52	
	1.1	13.4	1.6	
	7.39	8.9	1.02	
	3.44	15.9	1.85	
	1.78	4.5	1.22	
	11.79	19	2.12	
	1.88	17.8	1.68	

	0.87	16	1.68	
	2.83	13.9	1.49	
	2.63	18.9	1.88	
4/6/2010	10.66	15.3	1.5	7
9/4/2014	1.86	10.9	1.22	2
11/2/2014	9.282		2.41	4
12/30/2014	0.42	18.8	1.79	5
12/6/2010	3.82	13.7	1.83	10
6/29/2016	0.737	13.7	1.75	3
4/6/2017	0.348	13	1.35	3
5/18/2017	0.252	9.7	1.37	3
8/8/2010	2.93	11.4	1.68	3
5/7/2013	2.5	15.7	1.7	9
	1.23	17.7	1.31	
6/24/2013	5.694	11.7	1.27	7
	2.52	7.6	1.18	
11/28/2011	3.01	10.4	1.47	25